Reproductive Biology and Control of *Mikania micrantha*

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Abstract

Mikania micrantha is a member of the family Asteraceae (Compositae). Its origin is in tropical Latin and South America. This species was introduced to Hong Kong probably in the late 19th century. Old specimens were identified as *Mikania guaco* Desc., but has been re-identified as *Mikania micrantha* H.B.K. by authorities in U.K. It is a vigorous weed climbing over trees and shrubs in many parts of Hong Kong from hilltops at 750m altitude to mangrove marshes at the sea level. Each inflorescence bears four flowers borne in a capitulum enclosed by phyllaries. Each flower is equipped with pappus hairs which facilitate the dispersal of its fruits.

Mikania micrantha poses the greatest threat to vegetation in the Mai Po Nature Reserve, it is found on the roadsides, fences, intermingling among vegetations and the mangrove areas. Mangel plant, *Kandelia candel*, along the bunds has been seriously affected. *Mikania micrantha* is a problem which is not going to go away. Local action is urgently required.

In order to study the control of *Mikania micrantha*, three aspects were studied: (1) its reproductive biology, (2) effect of sodium chloride on the germination of the seeds and (3) effect of sodium chloride on the plant. Sodium chloride is a natural component in Mai Po Marshes and thus can be explored as a herbicide in the mangrove areas.

In the study on reproductive biology of this weed, self-pollination, cross-pollination and apogamy fertilization of the flowers were conducted. Samples of flowers were studied from November to January. It was found that the percentage of fruiting of flowers for apomixis is 91% and for the untreated is 94%. Apomixis is regarded as the mode of reproduction in this weed.

In the experimental studies, sodium chloride at the concentration of 7 parts per thousand (ppt) could completely inhibit germination. The concentration of sodium chloride in sea water being 30 ppt. At concentrations of 3 ppt and 5 ppt sodium chloride reduced germination to 52% and 35% at 30°C respectively.

Plants of *Mikania micrantha* were treated by foliage-application and soil-application of sodium chloride. Sequences of the changes of symptoms occurred in the treated leaves were recorded. In order to increase the wetting effect and reduce the surface tension of sodium chloride solution for foliage-application, the detergent brand "Axe" at a concentration of 1.5% was added as a surfactant. The results showed that 1%, 3% and 5% of sodium chloride mixed with the surfactant would kill the leaves at day 7, 5 and 3 respectively, whilst application of 5% sodium chloride to the root would kill the plant at day 7. The detergent alone for foliage-application showed no phytotoxic effects. Mangrove plants, *Kandelia candel* and *Aegiceras corniculatum*, were found resistant to foliage-application of sodium chloride and the surfactant.

微甘菊的生殖方法及控制其生長的研究

微甘菊屬菊科植物，原產在拉丁及南美國家。最早可能在十九世紀引入香港，當時鑒定爲 *Mikania guaco* Desc.，後再被鑒定爲 *Mikania micrantha* H.B.K.。微甘菊在每年十月至翌年一月開花，花朵細小，每四朵花給葉狀苞包圍著成爲一個頭狀花序。果實有冠毛方便傳播。

生殖方式研究包括試驗其自花授粉、異花授粉及無融合生殖能力。無融合生殖實驗中見有 91%的花朵結爲果實。利用氯化鈉（sodium chloride）作爲除莠劑來控制。微甘菊生長的實驗顯示：氯化鈉濃度爲 3 ppt 及 5 ppt 時，微甘菊種子的萌發率爲 52%及 35%；在 7 ppt 時種子完全喪失萌發能力。通過在葉部噴射氯化納（加入少許洗潔精）的實驗顯示，1%，3%及 5%的氯化鈉分別在七、五及三天內使葉部死亡。而通過將這三個濃度的氯化納加入泥土中的處理則分別在十二、九及七天內使整株微甘菊植物死亡。

微甘菊於香港米埔自然保護區的爲患甚大，有很多植物都受影響，當中紅樹林植物水筆仔（*Kandelia candel*）及桐花樹 (*Aegiceras corniculatum*)都受影響。實驗結果証實氯化鈉可導致微甘菊死亡，但不影響水筆仔和桐花樹這兩種植物的生長 。

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Chapter 1 : Introduction

1.1 General background

Mikania is a member of the family Asteraceae (Compositae). There are over 250 species of *Mikania* in the world (How, 1984). *Mikania micrantha* H.B.K. 微甘菊, a wide-spread herbaceous climber weed, is the only species found in Hong Kong. It is uncertain when it was introduced, but it was found in the Hong Kong Botanic Gardens as early as in 1884. Formerly the species occurring in Hong Kong was identified as *Mikania guaco* Desc., but recent identification as *Mikania micrantha* follows reference to authorities in U.K.

Mikania micrantha is a plant from Latin and South America. It is widely found in Southeast Asia and in tropical and subtropical regions of the world . It seriously affects the plantation crops such as tea, teak, rubber and oil palm in many parts of the world. In Hong Kong, it spreads widely from high altitudes of around 700m to Mai Po Marshes at sea level.

The seeds of *Mikania* are wind borne and thus readily colonise disturbed ground. It is likely that former intensive agricultural practices in the New Territories kept it under control, e.g. through regular spraying and burning off in garden areas. In recent years there has been a great reduction in such farming as land is increasingly used for old car

dumps and also left fallow pending road building etc. This has provided apparently ideal conditions for *Mikania* in the lowland in the New Territories. Once it has become established it quickly spreads both vegetatively and through seed dispersal.

Mikania micrantha causes serious ecological and environmental impacts adversely affecting (a) the growth of trees such as bamboos, mangroves, sapium, casuarina, and macarange and (b) wildlife ecology including some migratory birds which are unable to build their nests on shrubs and trees due to weed problem (Goh & Wong, 1994).

1.2 Morphology:

Mikania micrantha is a perennial herbaceous climbing vine. The leaves are opposite, green, petiolated, ovate, triangular, cordate, toothed irregularly along margins, with reticulate venation (Swarmy & Ramarkrishnan, 1987d; Figure 1.01).

Four flowers of *Mikania micrantha* are born in each inflorescence of capitulum (Figure 1.02) attached to a peduncle which arises from each node. Each inflorescence is subtended by large phyllaries. Each flower is usually 3 mm long and each has a corolla, gynoecium and androecium. Each single flower is actinomorphic, bisexual with the floral formula of: $\circledast\ \overset{\uparrow}{\varphi}\ \overset{\frown}{C_{(5)}}A_{(5)}\underline{G}_2$

The calyx is reduced to pappus hairs which would facilitate the dispersal of fruits by wind. The corolla is fused into a tube enclosing the gynoecium and androecium.

The androecium consists of five stamens. The anthers are opaque and white in colour. The filaments are attached to the corolla tube and the anthers are connate to form a whorl.

The gynoecium consists of a stigma, a style and an ovary. The stigma, which is the site for the reception of pollen grains, is bistigmatic with two flattened surfaces. The style is long and slender to extend the stigma out of the perianth. The ovary is inferior and bears one ovule (Figure 1.03) with basal placentation. After fertilization, the white ovule turns into a black seed and becomes hardened.

The fruit is blackish and hardened forming a black solid structure, tapering at both ends. Usually, the top of the fruit coat is attached by the pappus hairs which facilitate the dispersal of fruits and seeds by wind.

1.3 Problems of *Mikania micrantha* in Mai Po Nature Reserve:

Mai Po (Fig. 1.01) is the reserve area for migratory birds and natural vegetations. The wetlands around the Mai Po Marshes and Inner Deep Bay in the northwestern corner of Hong Kong, have been known as a haven for migratory birds for many decades. It was recognized as protected area in 1976, when it was designated as a Site of Special Scientific Interest. In 1984, World Wide Fund (Hong Kong) began to take over active management of the reserve for education and conservation. In 1995, 1,500 ha of wetlands around Mai Po and Inner Deep Bay was formally designated a Wetland of International

around Mai Po and Inner Deep Bay was formally designated a Wetland of International Importance under the Ramsar Convention. Apart from birds, the reserve has many other features of importance. The heart of the reserve is made up of 24 traditionally operated shrimp ponds (locally called *gei wai*), which are now the only such ponds in Hong Kong.

In Mai Po Marshes, which is managed by the World Wide Fund (HK) as a reserve area for birds and mangel vegetation, *Mikania micrantha* can be found growing on the roadside, fences and among such plants as *Melia azedarch* 苦 練 , *Panicum maximum* 大黍 , *Lantana camara* 馬纓 丹 and *Kandelia candel* 水 筆 仔. It is also extensively colonizing in the mangrove area of the Mai Po Marshes.

Since *Mikania micrantha* is a noxious weed which competes with other plants for sunlight, water, oxygen, carbon dioxide and even space, control of this weed is urgely needed in order to maintain the natural beauty of countryside, to prevent obstructions to the roadsides and fences, and to prevent it from competition of growth with desirable plants. For this reason, Mr. David Melville (1987), Director, WWF (Hong Kong), complained:

> "At Mai Po *Mikania micrantha* poses the greatest threat to vegetation in the area. Already areas of *Lantana camera* and *Phragmites communis* have been killed due to swamping and growth of mangroves (*Kanedlia candel*) along the bunds has been seriously affected. Shoot growth of *Mikania micrantha* is rapid in summer and it is quickly spreading along the bunds."

a radio programme broadcast on 11 and 12 October 1987 a spokesman stated that AFD would "approach the universities to see whether they have resources to establish certain trial plots or carry out certain control works in the control of *Mikania*." Up to 16 October 1987 the two universities had not been approached by AFD and it is unclear what will happen if the universities do not take up the project but it appeared from the radio programme that Government would no longer concern itself with the matter."

"In the meantime WWF HK continues to carry out control works on its landholding at Mai Po but clearly is unable to do anything on land outside its control—sadly the Government appears to be unwilling to take up the challenge."

"WWF HK has made lengthy discussions with the Agriculture and Fisheries Department (AFD) for over two years but little action has resulted and it is clear that there are difference in opinion among Departmental staff regarding control measures. It has been reported recently that AFD has limited staff and funds for work on *Mikania* control but that the Department has not made any approach to Government for additional support since it is assumed that funds will not be forthcoming."

"*Mikania micrantha* is being a problem which is not going to go away. Local action is urgently required at Mai Po but a long-term action plan is required for the whole territory."

Since the spreading of *Mikania micrantha* in Mai Po is so rapid and its effect is so serious, it has to be controlled and new control methods have to be discovered.

1.4 To further assess the problem of Mikania micrantha in Mai Po :

Interviews were made with the officers in Mai Po. They included Dr. Llewellyn Young, Mai Po Reserve Manager, on 23rd June 1999 and Mr. Wong Kam Moon, Gei Wai Officer, on 14th June 1999.

According to the two Officers, *Mikania micrantha* is found to colonize in a majority of the reserve area. The distribution of *Mikania micrantha* is mainly on the western border of Mai Po, a main road leading to the reserve area and on both sides of bunds among Gei Wai (traditionally operated shrimp ponds). The growth of *Mikania micrantha* is fast and it spreads rapidly. *Mikania micrantha* grows faster in summer and more slowly in winter.

Mikania micrantha grows well on all kinds of vegetations in Mai Po. They are spreading from the land vegetations to the mangrove plants. However, the growth of *Mikania micrantha* in the mangrove region is of lesser extent than that of the land. The growth of *Mikania micrantha* among *Kandelia candel* is extensive.

Various control measures including hand pulling, machinery and herbicides had been used without much success. The use of fire was of lesser extent. There was the spraying regularly in the land areas and hand pulling of the weed in the mangrove areas since the use of herbicides in the mangrove areas would be harmful to both the fauna and flora there.

Previous Studies:

Previous studies had been done on the study on the life cycle and the response to herbicides of *Mikania micrantha* (Hu & But, 1994). Seed germination of *Mikania micrantha* reached 95.3% within 6-8 days. Germination percentage was affected by temperature. At 5°C , only 0.33% of seeds germinated in 7 days. At 15-30°C, the germination percentage reached 83.7%. However, at 40°C, only 1% of seeds germinated. Light was found to have effects on seed germination. Under complete darkness, 35.3% of seeds germinated, and the growth of plumules and radicles were retarded. Upon exposure to light of 6 hours or more, 70% of seeds germinated and the growth of plumules and radicles was extensive. Four herbicides: Roundup, Bentazon, Tordon and Ronstar were studied. They showed inhibitory effects on seed germination and seedling growth, but Bentazon and Tordon had stronger inhibitory effects on *Mikania micrantha.*

The fungal pathogens for biological control of Mikania micrantha was reported by Barreto and Evans (1995). Field observation in Brazil indicated that *Basidiophora montana* has potential as a classical biological control agent of *Mikania* in the Old World subtropical or montane climates, whilst *Mycosphaerella mikania-micranthae* and the microcyclic rust *Puccinia spegazzinii* appear to be equally damaging to *Mikania.* In foreign researches, mycoherbicide research and development over the past two decades has led to the commercial use of several indigenous plant pathogenic fungi for the control of various weeds. In preliminary survey, several pathogenic fungi were found on the *Mikania* weed, causing leaf spot and leaf blight disease. Although the disease observed

were not severe, the sources of pathogenic fungal strains should be further evaluated for the potential development of mycoherbicide to control the weed. Interest has been shown in the potential biocontrol strategies of *Mikania* in Hong Kong (Goh & Wong, 1994), despite several efforts attempted to solve this weed problem, an effective control strategy, either chemical or biological, with careful consideration of preventing environmental pollution, is still in great demand.

Research by the Commonwealth Agricultural Board (CAB) has identified the insect *Liothrips mikaniae* as a promising natural enemy for the control of *Mikania* (Melville, 1987). Preliminary laboratory tests by CAB in Trinidad indicated a high level of host specificity for *Mikania micrantha.* It is evident that what is needed is a cheap method of control with widespread impact. *Liothrips mikaniae* would appear worthy of consideration. Since June 1987 WWF (HK) has suggested to AFD that trials for food plant specificity should be conducted in Hong Kong to determine whether *Liothrips* would be suitable for control of *Mikania*. It is not clear, however, whether or not *Liothrips mikaniae* would restrict itself to *Mikania micrantha* in Hong Kong.

Various control measures had been done in Hong Kong and overseas (Melville, 1987). WWF HK has done intensive hand weeding with limited success. Burning off in winter is not successful since the plant is deep rooted and the roots are not killed, allowing regeneration from lower nodes. For the herbicides, it is found that *Mikania* is moderately susceptible to 2,4-D. Trials by WWF HK at Mai Po have demonstrated that glyphosate is largely successful, providing that follow-up spraying is done and the dead material is

removed by hand. Trials by Agriculture and Fisheries Department at Mai Po using the selective herbicides were unsuccessful, but no surfactant was added to the spray and no follow-up spraying was done.

Dodder *Cuscuta* grows well on *Mikania* in Hong Kong but has no apparent effect on the host plant other than local discoloration of the leaves. Dodder have been used in Sri Lanka to suppress the spread of *Mikania* from wasteland to tea plantation but it is not a selective parasite and thus must be used with extreme caution.

It has been suggested by AFD that tree planting is useful since a dense canopy will block out the light and prevent growth of *Mikania*. This may work, but has yet to be demonstrated in Hong Kong, and *Mikania* normally does occur as ground cover in rubber plantation in tropical areas. It is likely that the only local tree species useful is *Macaranga tanarius* (Melville, 1987). If planted densly this tree prevent growth of many other herbs through shading and this may not be desirable. Furthermore, extensive planting would have considerable landscape implications which might be considered undesirable.

1.6 Experimental Aims:

The present research study focused on the reproductive biology and a new control method for *Mikania micrantha*. For the reproductive biology, attention mainly focused on the mode of successful pollination of this weed, to see if it is self-pollinated, cross-pollinated or apomitic. For the control of this weed, particularly in Mai Po Marshes, sodium chloride was used as the control agent on the germination of fruits and on the growth of young and adult plants.

The research areas were divided into three parts:

1. To study the mode of reproduction of *Mikania micrantha*,
2. To study the effect of sodium chloride as a control agent on the germination of seeds of *Mikania micrantha*, and
3. To study the effect of sodium chloride as a control agent on the growth of young and adult plants of *Mikania micrantha*.

Fig. 1.01. Growing plants of *Mikania micrantha*. The plants intermingle among other vegetations. The leaves are ovate, opposite and the inflorescences of flowers are born on peduncles.

Fig. 1.02. A capitulum of flowers of *Mikania micrantha*. Four flowers are enclosed by phyllaries to form a capitulum of flowers (25X).

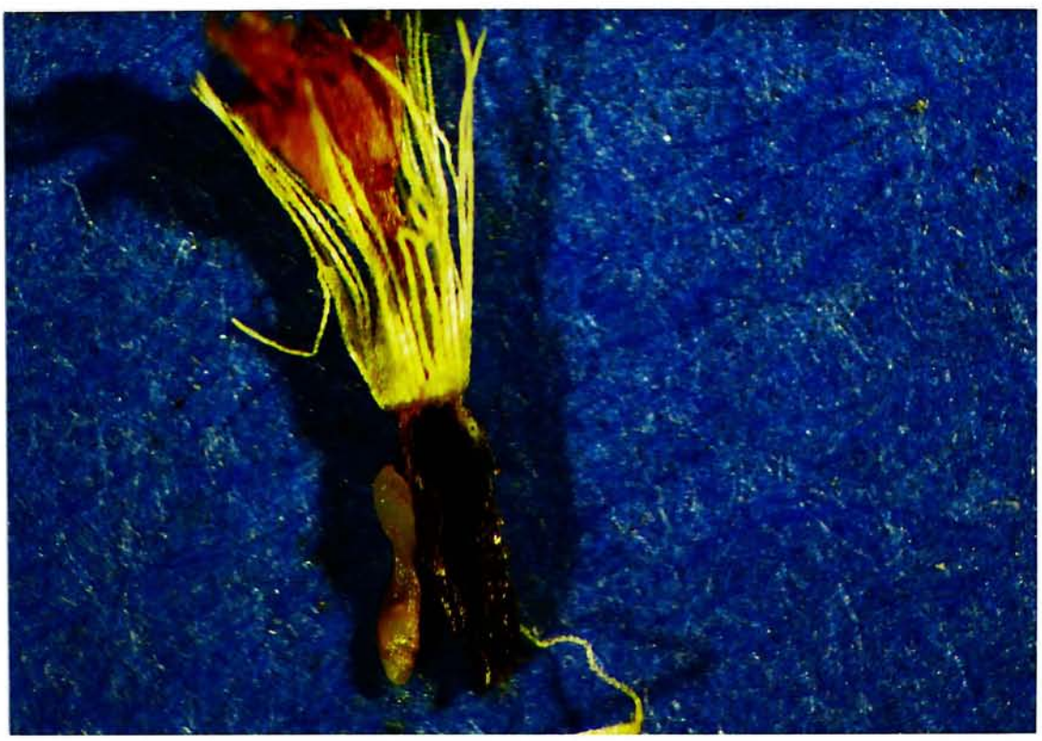

Fig. 1.03. Dissection of a single flower of *Mikania micrantha*. A single ovule (white colour) is pulled out from the ovary (30X).

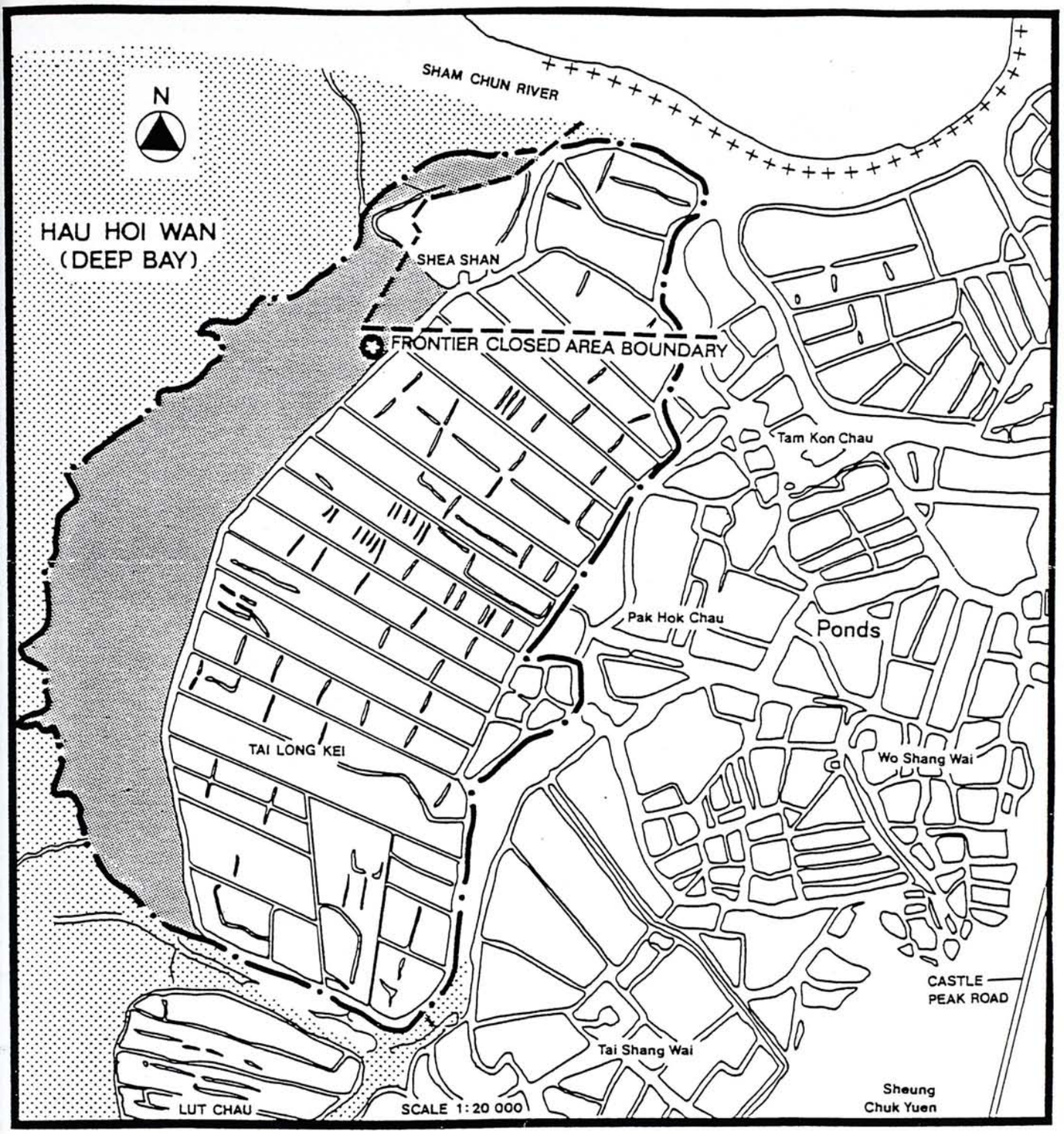

Fig. 1.04. Map of nature reserve of Mai Po in Hong Kong. (Circled by dark broken line)

Chapter 2 : Mode of reproduction of *Mikania micrantha*

2.1 Introduction

Different adaptations to particular methods of pollination, especially the frequently highly-developed specialisations for distinct pollinating animals as well as the establishment of special methods of fruit and seed dispersal are not usually confined to individual characters (Faegri & Pijl, 1966), but concern the totality of the floral organs. Pollination adaptations include flower shape, colours and scent, flowering time and duration, the order of dehiscence of the anthers, the morphology of the gynoecium as well as the arrangement of flowers themselves and positions on plants (Weberling, 1981).

Asexual reproduction can take place in the floral region leading to asexual development of seeds (Sven & Jerling, 1992) and also in the vegetative region by means of stolons, bulbils and other organs of vegetative reproduction (Weberling, 1981).

For asexual reproduction, this guarantees genetic stability which permits a population to survive in a particular locality. On the other hand, sexual reproduction which has a certain degree of genetic plasticity would maintain adaptability to changes in the environmental conditions through possible recombinations of old and new characters (Weberling, 1981).

A study of the reproductive mode of *Mikania micrantha*, whether apomixis, self-pollination or cross-pollination, may allow us to see if it could be controlled at the reproductive stage. It is particularly significant if the extensive colonization of *Mikania micrantha* can be inhibited at the early stage of establishment of the plant.

2.2 Literature Review

2.2.1 Pollination

There are usually three types of pollination methods adopted by the plants according to the floral structures, external environments and even the vectors. They are self-pollination, cross-pollination and apoximis. Self-pollination is the transfer of pollen from the anther of one flower to the stigma of the same flower. It is the stigma that acts as the receptive surface for the pollen grains and hence enhance the success of pollination. In environments poor in pollinators and of unfavorable conditions, self pollination is often the only possible means of sexual reproduction. The flowers are often inconspicuous, and often lack scent and nectar (Lloyd, 1992). Cross-pollination is the transfer of pollen from the anther of one flower to the stigma of another flower of the same species. Flowers are usually more conspicuous and scent and nectar are often present (Lloyd, 1992). Apomixis is the formation of seed without the sexual fusion of gametes (Sven & Jerling, 1992). There may or may not be any transfer of pollen grains from an anther to a stigma whether in the same flower or different flowers of the same plant or another plant of the

same species. It involves the formation of fruit from ovary and seed from somatic cells directly.

2.2.2 Sexual Reproduction

In sexually reproducing angiosperm, megaspore mother cells divide meiotically to form four haploid megaspores. One of them enlarges and gives rise by mitotic divisions and differentiation to the female gametophyte or embryo sac. Usually, the embryo sac has eight nuclei but only seven cells, as the central cell contains two polar nuclei. The "egg apparatus" with the egg cell surrounded by two synergids is situated at the micropylar end of the embryo sac, at which end the pollen tube enters. The three antipodal cells, usually short-lived, occur at the chalazal end (Lloyd, 1992; Sven & Jerling, 1992).

The male gametophyte of angiosperms is still more reduced. In anthers, microspore mother cells (pollen mother cells) enter meiotic division, each producing four reduced microspores. In each microspore, the nucleus divides mitotically to form a two-celled pollen grain with a vegetative cell and a generative cell. The generative cell divides once more and gives rise to two sperm nuclei. The last-mentioned division usually takes place after pollen germination.

Pollen grains germinate on the stigma. The pollen tubes grow down the style, and enter the ovule through the micropyle opening. Two male nuclei pass into the embryo sac

after penetration of the pollen tube into the degenerating synergid. One of the male nuclei fertilizes the egg cell to form the zygote. The other male nucleus fuses with the polar nuclei, forming a triploid cell which typically produces the endosperm, functions as nutritive tissues. In the embryo, developed from the fertilized egg cell, the unreduced chromosome number would be restored. This double fertilization is unique to the angiosperm.

2.2.3 Asexual reproduction

Under asexual reproduction, there are the vegetative propagation and apomixis. The vegetative propagation is the formation of offsprings through the somatic cells, such as the stolons, rhizomes and runners. However, in the flowers in the floral region, the type of asexual reproduction in angiosperms is apomixis.

Apomixis is the formation of seed derived from somatic cells in the female sex organ without the sexual fusion of male and female gametes. Sometimes modifications of reproductive behavior occasionally observed in sexual species include unreduced embryo sacs, pathenogenetic development of reduced as well as unreduced egg cells, and the presence of nonfunctional aposporous embryo sacs (Sven & Jerling, 1992).

2.3 Materials and methods

2.3.1 Field Work

Sites of places with the growth of *Mikania micrantha* were found on the campus of the Chinese University of Hong Kong. The sites were selected for easy accessibility, for regular visits and for the observation of the growth of large populations of *Mikania micrantha*. The sites should not be on the hillsides, difficult for access, obstructed by the iron fences or too much intermingled with mixed vegetations. Sites were selected on the roadside leading to the Marine Science Laboratory near the Tolo Harbour. The sites were quiet and would not commonly be disturbed by human activities. The chosen sites were surrounded by strings and wooden frames as a restricted area, to prevent disturbance.

2.3.2 Treatment of flowers

(a) Apomixis

Totally 60 fascicles of capitula which totally accounted for 3,170 single flowers were chosen at the site for testing of apomixis. For each single flower, a scissor was used to cut off the upper part of the flower including the upper corolla, the anther, style and stigma but just leaving the lower part of remains of perianth and the ovary. Each fascicle of capitulum of flowers treated were covered by a small white cotton bag with a knot made to be squeezed at the bottom. Experimental site set up for the treatment of flowers of *Mikania micrantha* was shown in Figure 2.08. In order to prevent the entry of pollens

from outside due to handling, the knot should be squeezed completely to cover the whole fascicle of capitulum of flowers. The fascicle of capitula were then marked numerically as labels. The treated flowers were then subjected to the natural environment of temperature, sunlight, photoperiod, atmospheric pressure and wind velocity.

(b) Self-Pollination

Totally 200 single flowers were chosen at the site for study. Each single flower was obtained by the removal of all other flowers in a fascicle of capitulum (using a scissor to remove wholly the other flowers in a fascicle of capitulum but just leaving one flower). Then the single flower was covered by a small white bag with a knot at the bottom and subjected to the natural environment.

2.3.3 Untreated flowers

50 fascicles of capitula of flowers were selected without treatment, i.e. without either removal of any parts of the flowers or the coverage by small white cotton bags onto the flowers. Plastic strips of labels were used to label the selected fascicles of capitula of flowers. They were left undisturbed and exposed to the natural environment.

2.3.4 Data Record

Sites were set up in late October and the flowers were observed once every two days to look for any formation of fruits from the flowers. The fruits were recognized in the ovary parts which became hardened and blackened solid structures whilst the ovary was greenish-white in colour. Careful removal of the small white cotton bags for the treated flowers in apomixis and self-pollination. Then it was followed by the observation and data recording of any fruits formed from the flowers. The total number of flowers that formed fruits in each fascicle of capitulum as a fraction of the total number of flowers in the fascicle of capitulum was recorded and the percentage of fruiting was calculated. Then the treated flowers were covered again by the small white bags with squeezing the knots at the bottom of the bags to prevent the introduction of any pollen grains from outside due to handling.

For apomixis and untreated one, percentage of fruiting is calculated as:

$$\text{Percentage (\%)} = \frac{\text{Total no. of flowers form fruits}}{\text{Total no. of flowers in fascicles of capitulum studied}} \times 100\%$$

The overall percentage of fruiting of flowers on a particular date was calculated as:

$$= \frac{\sum(\%\text{ of fruiting of flowers})}{\text{total no. of flowers studied}}$$

For the treatment of self-pollination:

$$\text{Overall percentage} = \frac{\text{Total number of fruiting flowers individually}}{\text{sample size of flowers (200)}} \times 100\%$$

2.4 Results:

The results of success of forming fruits in *Mikania micrantha* were reflected in the treatment of apomixis, self-pollination and untreated flowers. The significant result is simply reflected by the number of the fruiting flowers and the percentage of the fruiting flowers in a total number of the study samples. The fruiting flowers (Figure 2.09) are yellow and dry, bearing pappus hairs for the dispersal of fruits (Figure 2.10). Results of treatment of apomixis, self-pollination and untreated are summarized below.

2.4.1 Treatment of apomixis

Treated fruiting flowers of *Mikania micrantha* in apomixis was shown in Figure 2.11. The number of fruiting flowers were recorded in the treatment of flowers on apomixis. The number was recorded on the whole fascicle of capitulum as a unit. The total number of flowers in a fascicle of capitulum was recorded and the total number of fruiting flowers in a particular fascicle of capitulum was also recorded. It is shown in Table 2.01, 60 fascicles of capitula were recorded with each individual with particular number of fruiting flowers over the total number of flowers in a fascicle of capitulum on a particular date of treatment. The "0" indicated that there was no fruiting occurred in the fascicle of capitulum and the first number indicated the number of flowers which formed fruits. It was found that when the flowers form fruits, usually the whole fascicle of capitulum form fruit altogether at a certain date. It showed that a particular fascicle of capitulum matured

and developed at a similar rate whilst different fascicles of capitula matured and developed at a different rate.

The number of fruiting flowers and the percentage of fruiting on different days of treatment of flowers on apomixis are shown in Table 2.02 and in Figures 2.01 and 2.02. For the 60 fascicles of capitula of flowers studied for apomixis, there were a total of 3,170 flowers. This was found that the initial number of the fruiting flowers was very low, only 166 of the 3,170 flowers, accounting for a percentage of 5.23%. Then there was a gradual increase in the number and the percentage of fruiting with days of treatment of flowers. The increase was changed from slow to fast and then slowed down when the percentage of fruiting was over 70%. A maximum percentage of fruiting of 91.1% was recorded on January 15, 1998. From December 24 to January 9, there was a rapid rise in the number of fruiting flowers and the percentage of fruiting increased from 587 to 2372.

2.4.2 Treatment of self-pollination

The number of fruiting flowers and the percentage of fruiting on different days of treatment of self-pollination is shown in Table 2.03 and in Figures 2.03 and 2.04. A total of 200 flowers were treated for self-pollination. The initial number of fruiting flowers was 10 out of 200 which accounted for 5% of fruiting. Whilst there was a rapid rise from December 22 to December 28 to 36% . However, there was then a slow down in the percentage of fruiting from December 28 to January 15. A maximum of 56% fruiting was concluded.

2.4.3 Untreated flowers:

The number of fruiting flowers and the percentage of fruiting on different days of experiment on the untreated flowers is shown in Table 2.04 and in Figures 2.05 and 2.06. This was shown that the percentage of fruiting of the untreated flowers initially was very low. Then it was followed by a slow, rapid and later slow increase in both the number and the percentage of fruiting. The maximum percentage of fruiting was found on January15 with 94%.

The percentages of fruiting on different days of experiment with apomixis, self-pollination and untreated flowers are shown in Table 2.05 and in Figure 2.07. It is found that the pattern of increasing in number and percentage of fruiting in the apomixis, self-pollination and untreated followed a similar pattern. The fruit rate was first slow, then fast and finally slow pattern. Whilst the initial percentage of fruiting on the record date in three cases was very low. However, the maximum percentages of fruiting on apomixis and the untreated were similar and the results followed a similar pattern. For the self-pollination, the maximum percentage of fruiting at the maximum was of a lower percentage and the increase pattern is not so significant as that of the apomixis and the untreated one.

Table 2.01. Fruiting of flowers in each fascicle of capitulum in apomixis

umbel/ date	22/12	24/12	28/12	5/1	9/1	13/1	15/1
1	0/32	0/32	4/32	32/32	32/32	32/32	32/32
2	0/52	0/52	6/52	52/52	52/52	52/52	52/52
3	0/64	0/64	64/64	64/64	64/64	64/64	64/64
4	0/64	0/64	64/64	64/64	64/64	64/64	64/64
5	10/12	12/12	12/12	12/12	12/12	12/12	12/12
6	0/16	0/16	4/16	4/16	4/16	16/16	16/16
7	0/20	0/20	4/20	4/20	4/20	20/20	20/20
8	0/32	32/32	32/32	32/32	32/32	32/32	32/32
9	0/42	0/42	42/42	42/42	42/42	42/42	42/42
10	0/80	0/80	0/80	0/80	80/80	80/80	80/80
11	0/92	0/92	0/92	0/92	92/92	92/92	92/92
12	0/84	0/84	0/84	0/84	84/84	84/84	84/84
13	0/88	88/88	88/88	88/88	88/88	88/88	88/88
14	0/80	0/80	80/80	80/80	80/80	80/80	80/80
15	0/36	0/36	0/36	0/36	4/36	36/36	36/36
16	0/20	0/20	0/20	0/20	20/20	20/20	20/20
17	0/24	0/24	24/24	24/24	24/24	24/24	24/24
18	0/32	0/32	32/32	32/32	32/32	32/32	32/32
19	0/24	0/24	0/24	0/24	4/24	24/24	24/24
20	0/32	0/32	0/32	0/32	0/32	0/32	0/32
21	0/92	0/92	4/92	4/92	4/92	4/92	4/92
22	0/94	4/84	4/84	4/84	4/84	4/84	84/84
23	0/36	0/36	0/36	36/36	36/36	36/36	36/36
24	0/44	4/44	44/44	44/44	44/44	44/44	44/44
25	0/40	0/40	0/40	0/40	0/40	40/40	40/40
26	0/84	0/84	0/84	84/84	84/84	84/84	84/84
27	0/60	0/60	60/60	60/60	60/60	60/60	60/60
28	44/72	44/72	72/72	72/72	72/72	72/72	72/72
29	0/56	0/56	56/56	56/56	56/56	56/56	56/56
30	0/52	0/52	0/52	0/52	52/52	52/52	52/52

umbel/ date	22/12	24/12	28/12	5/1	9/1	13/1	15/1
31	0/84	0/84	0/84	0/84	84/84	84/84	84/84
32	0/92	92/92	92/92	92/92	92/92	92/92	92/92
33	0/64	64/64	64/64	64/64	64/64	64/64	64/64
34	0/84	0/84	12/84	20/84	84/84	84/84	84/84
35	64/64	64/64	64/64	64/64	64/64	64/64	64/64
36	0/80	4/80	16/80	16/80	16/80	16/80	16/80
37	0/56	0/56	24/56	24/56	24/56	56/56	56/56
38	0/24	0/24	0/24	0/24	0/24	24/24	24/24
39	0/68	12/68	12/68	12/68	68/68	68/68	68/68
40	0/72	4/72	40/72	40/72	40/72	40/72	40/72
41	0/16	0/16	0/16	0/16	0/16	0/16	0/16
42	0/20	0/20	0/20	20/20	20/20	20/20	20/20
43	0/24	0/24	0/24	0/24	24/24	24/24	24/24
44	44/44	44/44	44/44	44/44	44/44	44/44	44/44
45	0/56	56/56	56/56	56/56	56/56	56/56	56/56
46	0/84	12/84	40/84	40/84	40/84	40/84	84/84
47	0/24	0/24	0/24	0/24	0/24	0/24	0/24
48	0/44	0/44	0/44	44/44	44/44	44/44	44/44
49	0/64	0/64	0/64	64/64	64/64	64/64	64/64
50	0/72	0/72	72/72	72/72	72/72	72/72	72/72
51	0/60	12/60	12/60	12/60	12/60	12/60	60/60
52	0/72	16/72	16/72	16/72	16/72	72/72	72/72
53	0/12	0/12	0/12	0/12	0/12	12/12	12/12
54	0/24	0/24	24/24	24/24	24/24	24/24	24/24
55	0/42	12/42	12/42	12/42	42/42	42/42	42/42
56	0/60	12/60	12/60	12/60	60/60	60/60	60/60
57	0/72	12/72	72/72	72/72	72/72	72/72	72/72
58	0/36	0/36	0/36	36/36	36/36	36/36	36/36
59	0/44	0/44	0/44	44/44	44/44	44/44	44/44
60	0/64	4/64	4/64	64/64	64/64	64/64	64/64

Table 2.02. Number of fruiting flowers and percentage of fruiting of the treated flowers in apomixis study from 22nd December to 15th January. Total number of fascicles of capitula are 60 and total number of flowers are 3,170.

Date	Cumulative no. of fruiting flowers	% of fruiting of flowers
22/12	166	5
24/12	587	19
28/12	1218	39
5/1	1685	53
9/1	2372	75
13/1	2668	84
15/1	2887	91

Table 2.03. Number of fruiting flowers and percentage of fruiting of the treated flowers in self-pollination study from 22nd December to 15th January. Totally 200 flowers were used for study.

Date	Cumulative no. of fruiting flowers	% of fruiting of flowers
22/12	10	5
24/12	45	23
28/12	72	36
5/1	86	43
9/1	95	47
13/1	104	52
15/1	112	56

Table 2.04. Number of fruiting of flowers and percentage of fruiting of the untreated flowers from 22nd December to 15th January. Totally 50 fascicles of capitula were used for study.

Date	No. of fruiting fascicle of capitula	% of fruiting of flowers
22/12	2	4
24/12	10	20
28/12	18	36
5/1	26	52
9/1	38	76
13/1	43	86
15/1	47	94

Table 2.05. The percentage of fruiting of flowers from December 22 to January 15 in apomixis study, self-pollination study and the capitula left untreated.

	% of fruiting of flowers		
Date	untreated	apomixis	self-pollination
22/12	4	5	5
24/12	20	19	23
28/12	36	39	36
5/1	52	53	43
9/1	76	75	47
13/1	86	84	52
15/1	94	91	56

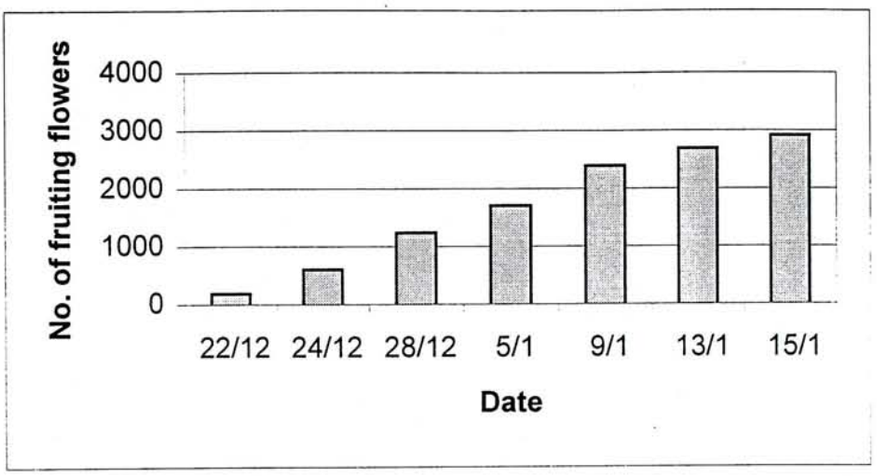

Fig. 2.01 Number of fruiting florets in apomixis study from 22nd December to 15th January.

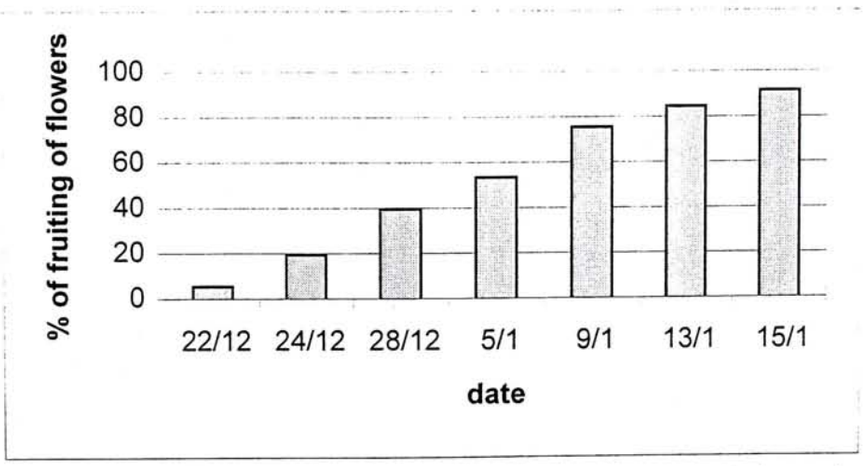

Fig. 2.02. Percentage of fruiting of flowers in apomixis study from 22nd December to 15th January.

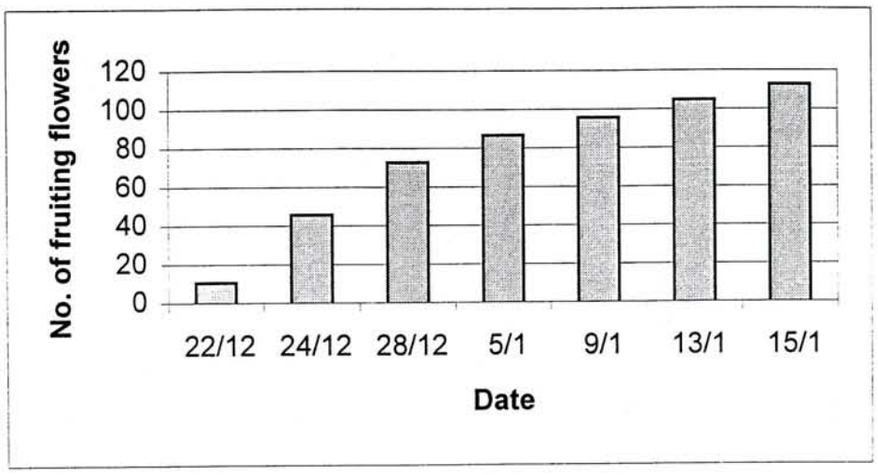

Fig. 2.03. Number of fruiting flowers in self-pollination study from 22nd December to 15th January.

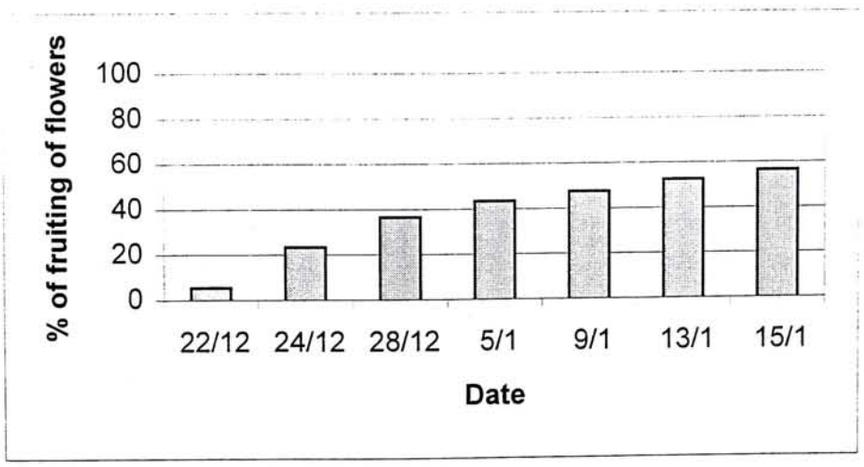

Fig. 2.04. Percentage of fruiting of flowers in self-pollination study from 22nd December to 15th January.

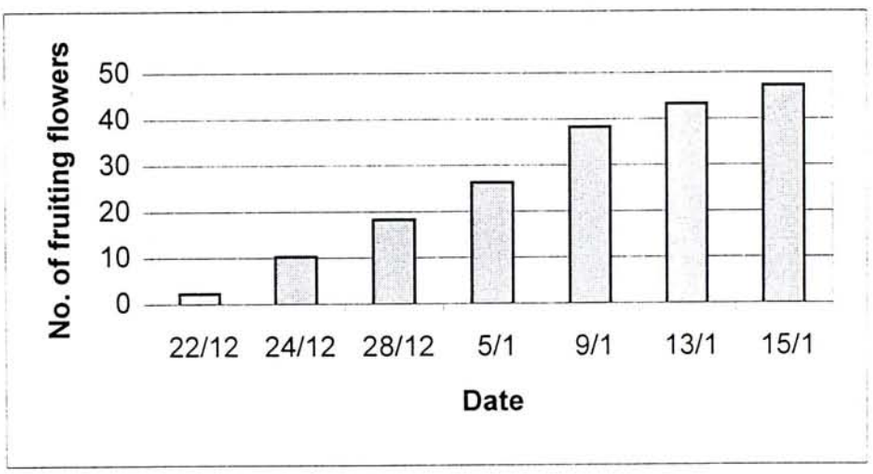

Fig. 2.05. Number of fruiting flowers from 22nd December to 15th January (Untreated).

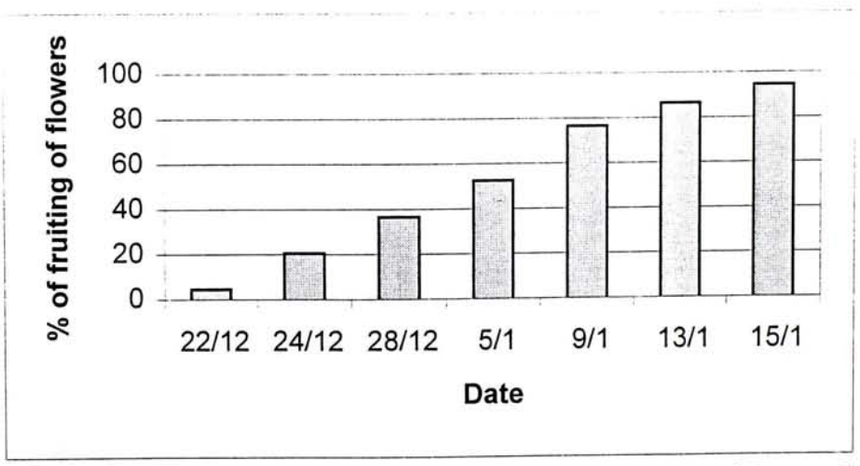

Fig. 2.06. Percentage of fruiting of flowers from 22nd December to 15th January (Untreated).

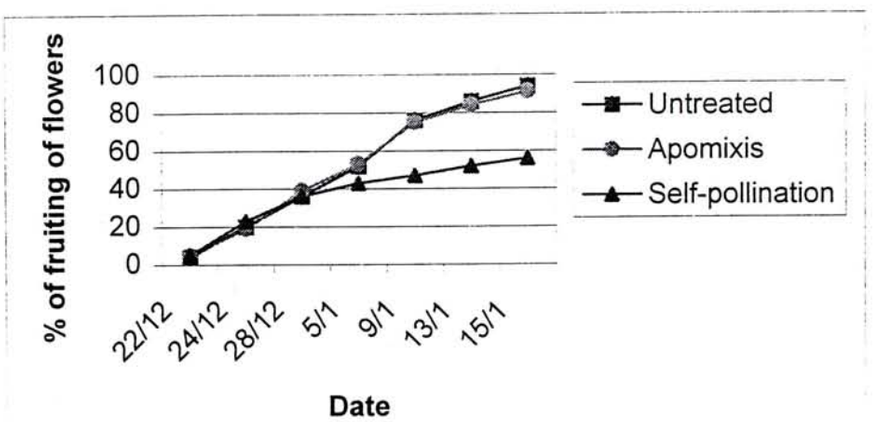

Fig. 2.07. Percentage of fruiting of flowers in Untreated, apomixis study and self-pollination study from 22nd December to 15th January.

2.5 Discussions

For the apomixis test, there was a high percentage of fruiting of flowers to about 90% on the record dates. The result is similar to that of the untreated, suggesting that the mode of reproduction of *Mikania micrantha* is apomixis. Since the untreated one acts as the control for the reference of the mode of natural reproduction in *Mikania micrantha.*

Apomixis is the formation of seed in *Mikania micrantha* without the sexual fusion of male and female gametes. *Mikania micrantha* bears a lot of flowers in each individual plant and the noxious, vigorous behavior is demonstrated by its success of reproduction to produce a lot of offsprings. Apomixis is a quick and energy-saving process which does not involve the maturation and fusion of both male and female gametes nor pollination agents, hence allowing its success of reproduction.

The pattern of formation of fruits in *Mikania micrantha* is similar. They are initially immature. Then, they will develop into fruits which are black, tough solid structures . In the study to check apomixis, an individual flower forms fruit and the whole fascicle of capitulum is normally forming fruits altogether. The fruits apparently developed at a similar rate. The genetic factors for the fascicle of capitula were probably the same; once they are exposed to the same orientation to the sunlight, same exposure to the wind direction and speed, same photoperiod, same height level from the ground, they are found to develop fruits at a similar rate.

During the whole process of experiment, several things were noted. First, the removal of the cotton bags for data record of the fruiting of flowers, then the covering again of the small white cotton bags for treatment should be careful because any careless handling of the small white cotton bags would introduce pollen grains from outside into the treated flowers. Since the pollen grains might be transferred onto the small white cotton bags and then transferred into the treated flowers upon handling, steady and careful removal followed by the complete coverage of the small white cotton bags onto the treated flowers were necessary. Second, the sites for the study should be left undisturbed without human disturbance. Finally, the data record of the fruits formed should be careful, since the fruits formed are only recognized by the colour and texture changes on the ovary parts of the flowers. Hence judgement on whether or not there was fruit formation had to be made wisely.

However, in the study of formation of fruits from the flowers in self-pollination, about 56% formed fruits from the flowers. If *Mikania micrantha* is reproduced by apomixis, then the percentage of fruiting of flowers in self-pollination should be theoretically to be similar to that of apomixis. However in the experimental data, it was found to be only 56%. One explaination is the fact that for the singly individual flower covered by a small white cotton bag, the size of the small white cotton bag was smaller when compared with that of the apomixis one, since it was used to cover only one flower instead of the whole fascicle of capitulum of flowers. Hence the passage of air, oxygen, carbon dioxide , sunlight, and wind might be limited and also the space was restricted

environment for the development into fruits. Hence the percentage of forming fruiting flowers were lower.

By studying the mode of reproduction *of Mikania micrantha*, we wished to find out how to control the weed at the reproduction level. If it were found to be cross-pollinated by vectors, spraying of some insecticides or insect repellents might prevent pollination, and hence might limit reproduction. This study showed the mode of reproduction of *Mikania micrantha* is apomixis; hence the prevention of flowering of *Mikania micrantha* and development into fruit is important. Future studies should explore the possibility of (a) introducing sterility in the plant, (b) preventing flowering in the weed, and (c) spraying of herbicides on flowering specimens during the flower season.

Fig. 2.08 Experimental site showing for the treatment of flowers of *Mikania micrantha*. Each whole fascicle of capitula of flowers was covered with a white bag.

Fig. 2.09. Fruiting flowers of *Mikania micrantha*. The flowers bearing the fruits are yellow and dry. Presence of pappus hairs for fruit dispersal.

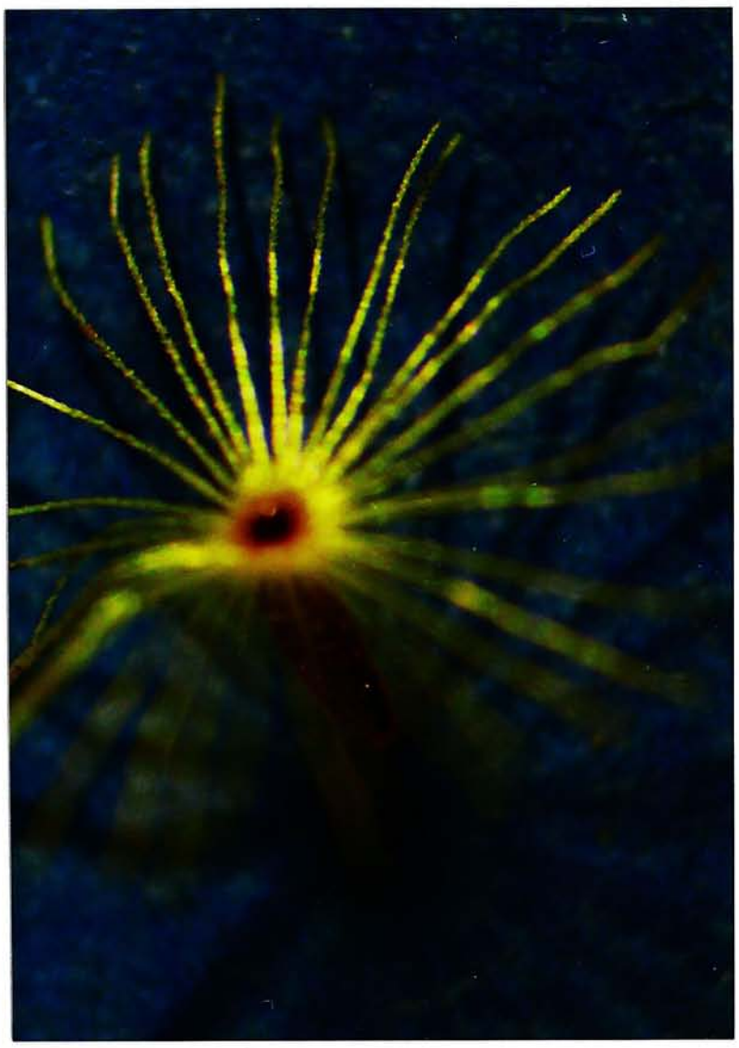

Fig. 2.10. Mature fruit of *Mikania micrantha*. Pappus hairs are attached to the fruit for dispersal.

Fig. 2.11. Treated fruiting flowers of *Mikania micrantha* in apomixis study. The upper corolla and the anther and the style parts were removed.

Chapter 3: Effect of sodium chloride on seed germination of *Mikania micrantha*

3.1 Introduction

The germination of seeds is mainly divided into three distinct stages. (Kipel & Galili, 1995). First, the breaking of dormancy of the seed. Dormancy of seed may be the result of seed coat impervious to gases or water; internal inhibitors; requirement for a proper thermoperiodic or photoperiodic treatment. This process involves the imbibition of water by seeds. The imbibition of water by seeds, the uptake is expressed as a function of osmotic potential (Ψ_π). Second, there is the onset of metabolic processes associated with germination. Finally, emergence of the radicles and subsequent growth and development of the seedling occur.

The onset of metabolic processes in seeds on imbibition involves the mobilization of the reserves in seeds (Kipel & Galili, 1995; Mohr & Schopfer, 1995). The reserves may be stored in cotyledons or endosperms. There are the hydrolysis of starch, protein and lipid by enzymes lipase, proteinase and lipase. The hydrolytic action of the enzymes required the presence of water. The hydrolysed product glucose which is the osmotically active product increases the uptake of water by seeds.

Fats are splitted into glycerol and fatty acids and the fatty acids dissimilate through β–oxidation to acetyl CoA. For the proteins, they are degraded into amino acids by hydrolytic action of proteases which are partly formed *de novo* during germination. The respiratory processes of the seed involves the synthesis of ATP and hence energy for the germination.

The germination process is followed by the synthesis of protein (Mohr & Schopfer, 1995) important for gene coding for enzymes involved in germination and reserve mobilization as well as for seedling growth.

Then it follows cell divisions and cell enlargement of the tissue propers. Firstly is the elongation of the hypocotyl and then the enlargement of the cotyledon. It is then followed by the radicle and plumule growth. The cell enlargement and division of the radicle causes the cracking of the seed coat to emergent the embryo.

The radicle of the embryo is developed into the future root of the plant. Plumule of the embryo is developed into the future shoot of the plant whilst the cotyledons of the embryo developed into the future leaves of the plant.

In the germination of seeds of *Mikania micrantha*, it is the emergence of radicle first, followed by the emergence of plumule and then the growth of two cotyledons.

3.2 Materials and Methods:

3.2.1 Experimental Set-up

Fruits of the *Mikania micrantha* were collected. They were recognized by the hardening and blackening parts on the ovary parts of the flowers. For each capitulum of flowers, each flower was separated. For each fruit, pappus hairs that were attached to the upper part of the fruit coat were removed. Then the fruits each with a seed inside were subjected to selection. Proper and healthy seeds were selected.

Petri dishes, each with a filter paper dotted with a red pen into a dotting pattern of 5 dots in a row and totally dotting of 5 rows into evenly distributed dots of 25 on each filter paper. The size of the filter paper was just fitted onto the petri dish. The set up of petri dish was repeated for the whole experiment.

Saline solution were prepared by adding sodium chloride (NaCl) into distilled water. Saline solution of concentrations 0.1%, 0.3%, 0.5%, 0.7%, 1%, 5% and 10% were prepared. Also distilled water for the experimental control set up was also prepared.

Saline solution of a known concentration was added onto each petri dish set up, until there was a complete moisturing of the filter paper. The filter paper should be completely moistened with the treatment solution but not leaving too much water on the dish. For each treatment solution or distilled water, eight petri dishes were prepared.

The dishes were labelled for particular solution of treatment. Then the selected seeds were placed onto the filter paper with the dotting pattern. Each seed was put on top of a single dot. Totally 25 seeds were put evenly distributed on each petri dish. The transfer of seeds were made by a forcep. Handling should be with care, not to press too hard, because excessive pressing would cause damage to the seeds. For each treatment solution, eight dishes of 25 seeds and totally 200 seeds were used for each treatment.

It was followed by daily addition of the treatment solution droplets and the experimental set up were left in the environmental condition of treatment. They were placed in the growth chamber of definite temperature: 30°C, 22°C and 12°C for study.

3.2.2 Data Record

Data sheets of table of 5X5 was used for recording the particular dish with specific locations of seeds. The data recorded included counting on each day the number of seeds that germinated. The first sign of germination was the cracking on the seed coat to expose the whitish underlying tissues.

Radicle and plumule length of a particular seed on particular dish was also recorded on the table. The radicle and plumule length were then recorded simultaneously by rulers of clear scales. The table was recorded daily for the three parameters.

For the table, the percentage of seed germiniation in each petri dish was calculated:

$G = N \div 25 \times 100\%$

G- % seed germinated

N- total no. of seed germinated in each dish

The set-up of the same treatment and conditions were repeated for eight times, which accounted for a total of 200 seeds for each treatment. The data for each set-up was recorded similarly and an average percentage of germination was calculated:

$Gn = (G1 + G2 + \ldots\ldots\ldots\ldots + G7 + G8) \div 8$

Whilst Gn is the overall percentage of seed germination for a particular treatment and G1....G8 are the percentage of seed germination for a particular treatment of each petri dish.

The radicle length of the particular seed on the petri dish was noted down and recorded in the table for the 25 seeds on a petri dish. Data for the other 7 dishes for the same treatment of seeds were similarly recorded.

$R = (\Sigma r)/N$

R- average radicle length

r- radicle length of a particular seed

N-total number of germinated seeds

For the plumule length, it is similar

$P = (\Sigma p) / N$

P- average plumule length

p- plumule length of a particular seed

N- total number of germinated seeds

Then a large table was prepared for recording the daily result of percentage of seed germination, radicle length and plumule length for a particular treatment solution.

Data were recorded daily until there was a slow down in the germination of seeds to a stop and there was a retardation in the growth of the radicles and plumules of the seeds.

3.3 Results

The percentage of seed germination, plumule length and radicle length of germinated seeds of *Mikania micrantha* with treatment of distilled water and different concentrations of NaCl at 30°C, 22°C and 12°C were shown in Tables 3.01, 3.02 and 3.03.

3.3.1 Percentage of seed germination

The percentage of seed germination was highest for those given distilled water (Table 3.01). It increased from an initial of 64% on day 3 to 80% at day 6 of treatment. For 1 ppt NaCl, the percentage of seed gemination was 70%, whilst for 3 ppt NaCl and 5 ppt NaCl, they were 52% and 35% respectively. At different concentrations of NaCl, the percentage of seed germination increased with increasing numbers of days of treatment of the seeds. Effects of different concentrations of NaCl on the percentage of seed germination at 30°C, 22°C and 12°C were shown in Figures 3.01 ,3.04, and 3.07.

The result showed that increasing the concentration of NaCl would decrease the percentage of germination of seeds. The result was obvious when comparing the 5 ppt NaCl with that of 1 ppt NaCl. Moreover, there was complete inhibition of seed germination for 7 ppt NaCl.

The rising patterns of seed germination for different concentrations of NaCl were similar and they were comparable to that of distilled water. The results of seed

germination of that of 1 ppt NaCl were similar to that of distilled water. Increasing concentration of NaCl from 3 ppt to 5 ppt and to 7 ppt, showed progressively significant inhibitory effect on the germination of seeds.

3.3.2 Plumule length

Plumule length of seeds treated with distilled water, increased from 0.18 mm on day 3 of treatment to 5.5 mm on day 6 of treatment (Table 3.01). The increase in the length of plumule was sharp, especially on day 4 of the treatment, then the increase was slow till day 6 of treatment.

It was shown that there was a decrease in the length of plumule of germinated seeds with increasing concentrations of NaCl (Table 3.01). Shorter plumule length paired with higher concentrations of NaCl treatment. For 1 ppt, the maximum plumule length was about 3.17 mm, for 3 ppt, it was 1.2 mm, but for 5 ppt, it was only 0.15 mm on day 6 of treatment. For 7 ppt, no germination of seeds and hence no plumule length was recorded.

The graphs of plumule lengths of germinated seeds against days of the experiment at 30°C, 22°C and 12°C were shown in Figures 3.02, 3.05 and 3.08 .

3.3.3 Radicle length

Radicle length of germinated seeds treated with distilled water, was 1.59 mm on day 3 of treatment and 12.5 mm on day 6 of treatment. The increase in radicle length was firstly at a slow rise and then a sharp rise especially on day 5 of treatment (Table 3.01).

It was shown that there was a decrease in the length of radicles of germinated seeds with increasing concentrations of NaCl (Table 3.01). Shorter radicle lengths matched with higher concentrations of NaCl on the same day of treatment of seeds. For 1 ppt, the maximum radicle length was 9.56 mm; for 3 ppt, it was 2.85 mm; but for 5 ppt, it was only 1.75 mm.

The graphs of radicle lengths of germinated seeds against days of the experiment at 30°C, 22°C and 12°C were shown in Figures 3.03, 3.06 and 3.09. It was a steady and fast rise upon the days of treatment. The slopes of the curves were sharper for the 1 ppt than that of 5 ppt NaCl indicating that the rate of increase in radicle length of 1 ppt treatment was larger than that of the 5 ppt treatment. For 7 ppt, no germination of seeds and hence no radicle length was recorded.

3.3.4 Effect of temperature on seed germination

Effect of seed germination with different concentrations of sodium chloride at 30°C, 22°C and 12°C were shown in Tables 3.01, 30.2 and 3.03 respectively.

It was found that temperature of 30°C and 22°C produced similar results of seed germination. For the distilled water, the percentage of seed germination were 80% and 75% for 30°C and 22°C. At 12°C, the percentage of seed germination was lower, only 23% on day 6 of treatment. For 3 ppt NaCl, the percentage were 52% and 56% at 30°C and 22°C but 10% at 12°C. The results were more significant for 5 ppt; the percentage were 35% and 32% at 30°C and 22°C but only 4% at 12°C. For 1 ppt, the percentage of seed germination were 70% and 64% at 30°C and 22°C but it was only 15% at 12°C.

For the germinated seeds, it was found that the development of the plumules and radicles were better at 30°C and 22°C. The plumule and radicle lengths were longer at 30°C and 22°C for all concentrations of sodium chloride whilst the lengths were shorter at 12°C. It was significant in the control treatment of 3ppt NaCl, with the plumule length of 1.2 mm at 30°C, 1.11 mm at 22°C but only 0.16 mm at 12°C. The radicle length of seeds treated with 3 ppt of NaCl was 2.85 mm at 30°C, 3.01 mm at 22°C but only 0.88 mm at 12°C.

Temp: 30°C

Days		dH_2O	1ppt	3ppt	5ppt	7ppt
3	G:	64%	58%	25%	15%	nil
	P:	0.18	0.12	0.07	0.05	
	R:	1.59	0.95	0.74	0.18	
4	G:	65%	60%	35%	28%	nil
	P:	2.05	1.25	0.48	0.12	
	R:	4.72	2.37	2.24	1.07	
5	G:	75%	66%	52%	32%	nil
	P:	4.35	2.35	0.86	0.15	
	R:	9.85	6.48	2.77	1.15	
6	G:	80%	70%	52%	35%	nil
	P:	5.5	3.17	1.2	0.15	
	R:	12.5	9.56	2.85	1.75	

G, P and R represent the percentage of germination, and plumule and radicle lengths of seeds

Table 3.01. Percentage of germination, and plumule and radicle lengths of seeds with treatment of different concentrations of NaCl at 30°C.

Temp: 22°C

Days		dH_2O	1ppt	3ppt	5ppt	7ppt
3	G:	40%	35%	21%	15%	nil
	P:	0.12	0.08	0.05	0.05	
	R:	1.21	0.12	0.68	0.12	
4	G:	55%	45%	38%	24%	nil
	P:	1.89	0.88	0.36	0.1	
	R:	3.68	2.15	2.11	0.98	
5	G:	75%	60%	48%	30%	nil
	P:	3.78	2.13	0.75	0.13	
	R:	7.56	5.89	2.79	1.12	
6	G:	75%	64%	56%	32%	nil
	P:	4.72	2.95	1.11	0.15	
	R:	9.83	8.33	3.01	1.67	

G, P and R represent the percentage of germination, and plumule and radicle lengths of seeds

Table 3.02. Percentage of germination, and plumule and radicle lengths of seeds with treatment of different concentrations of NaCl at 22°C.

Temp: 12°C

Days		dH_2O	1ppt	3ppt	5ppt	7ppt
3	G:	3%	2%	2%	0%	nil
	P:	0.03	0.02	0.02	0	
	R:	0.37	0.21	0.17	0	
4	G:	12%	8%	5%	3%	nil
	P:	0.25	0.05	0.02	0	
	R:	0.85	0.76	0.46	0.35	
5	G:	21%	12%	7%	3%	nil
	P:	0.48	0.27	0.17	0	
	R:	1.75	1.05	0.75	0.45	
6	G:	23%	15%	10%	4%	nil
	P:	0.52	0.33	0.16	0	
	R:	2.01	1.66	0.88	0.52	

G, P and R represent the percentage of germination, and plumule and radicle lengths of seeds

Table 3.03. Percentage of germination, and plumule and radicle lengths of seeds with treatment of different concentrations of NaCl at 12°C.

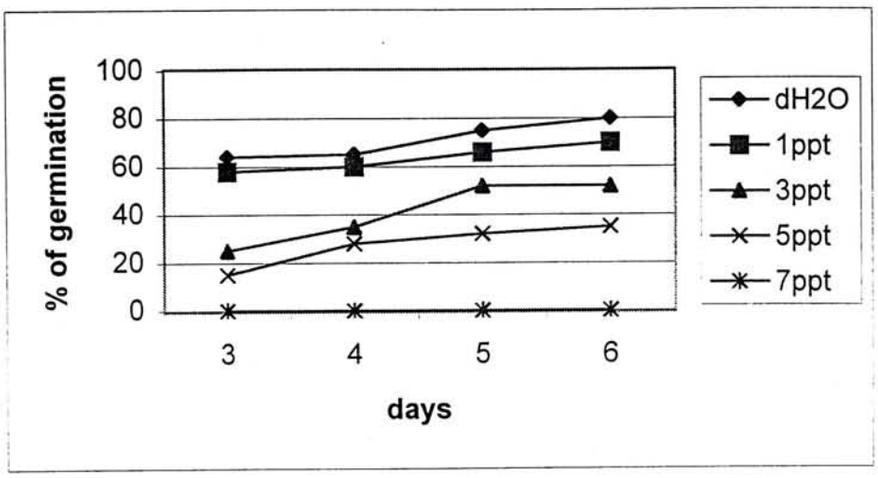

Fig. 3.01. Percentage of seed germination against days of treatment on different concentrations of NaCl at 30°C.

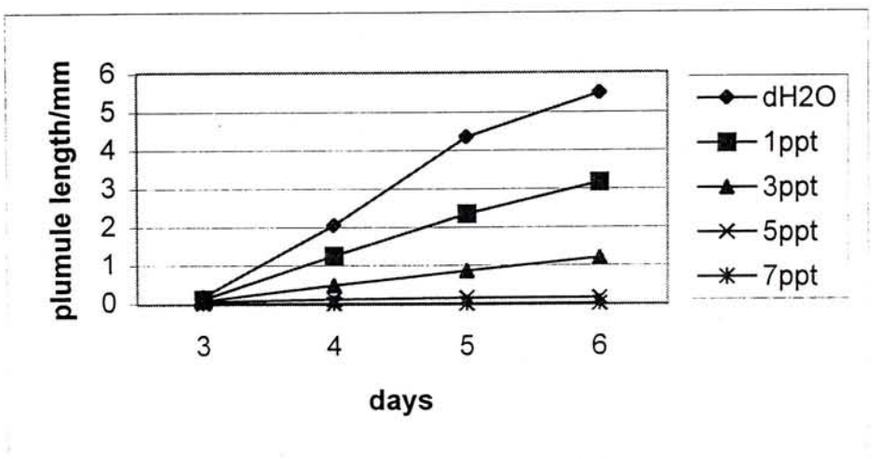

Fig. 3.02. Plumule lengths of seeds against days of treatment on different concentrations of NaCl at 30°C.

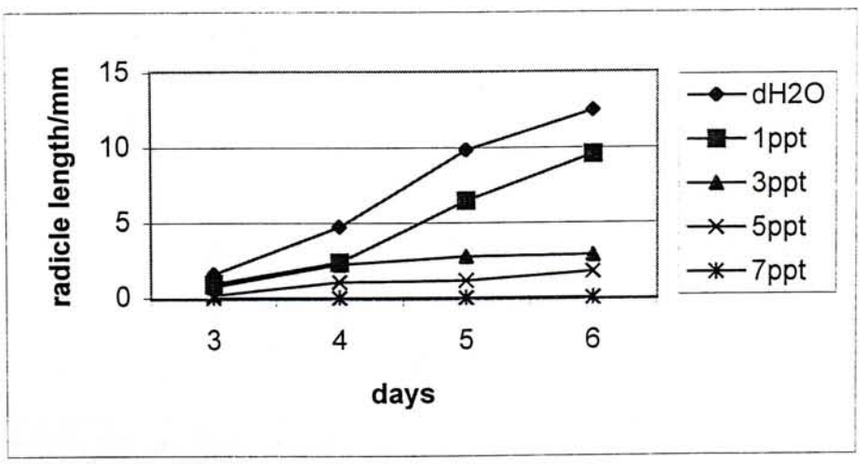

Fig. 3.03. Radicle lengths of seeds against days of treatment on different concentrations of NaCl at 30°C.

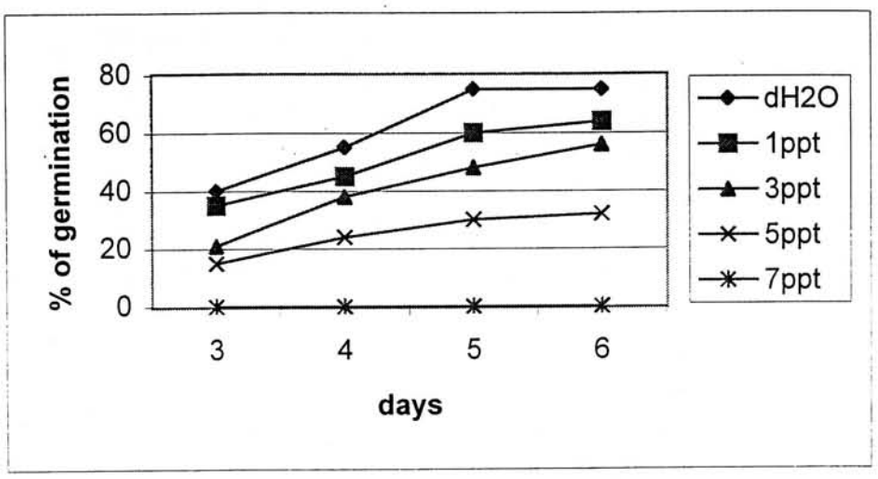

Fig. 3.04. Percentage of seed germination against days of treatment on different concentrations of NaCl at 22°C.

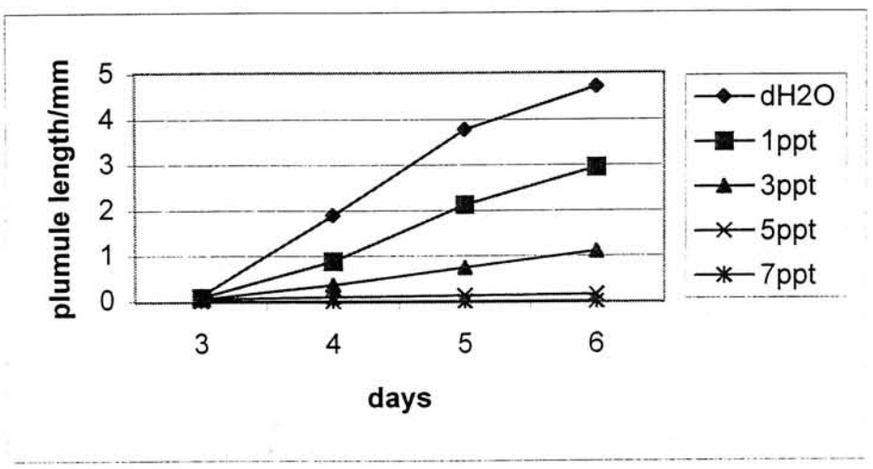

Fig. 3.05. Plumule lengths of seeds against days of treatment on different concentrations of NaCl at 22°C.

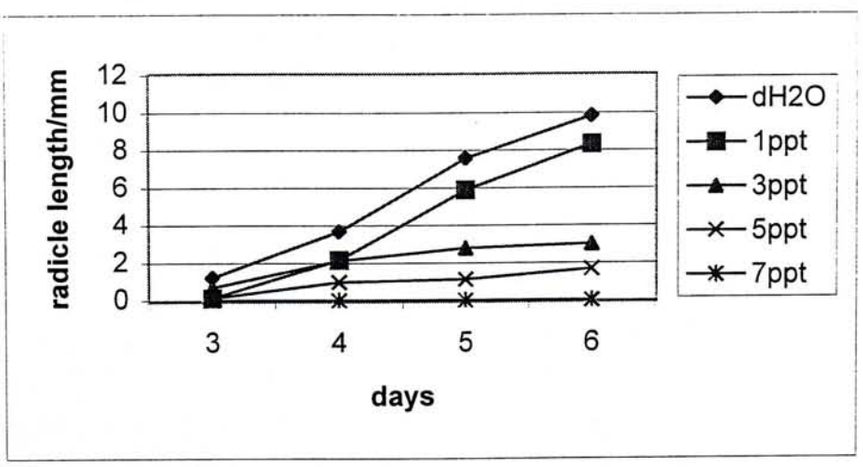

Fig. 3.06. Radicle lengths of seeds against days of treatment on different concentrations of NaCl at 22°C.

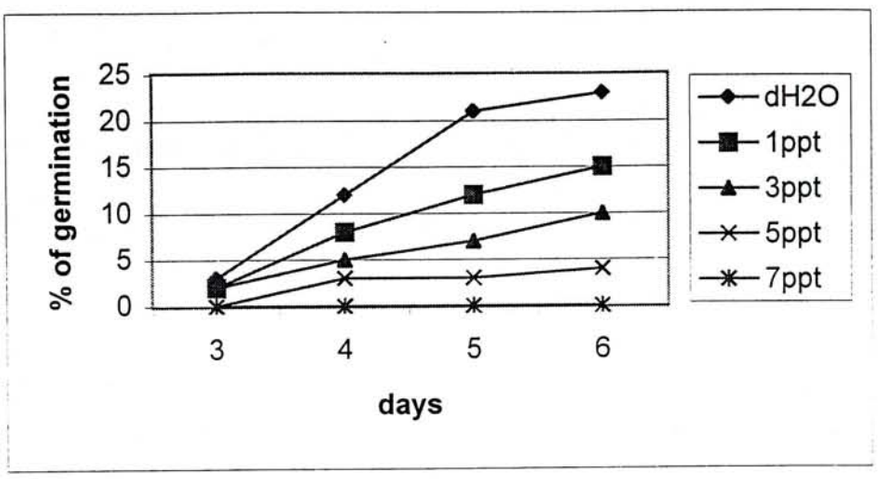

Fig. 3.07. Percentage of seed germination against days of treatment on different concentrations of NaCl at 12°C.

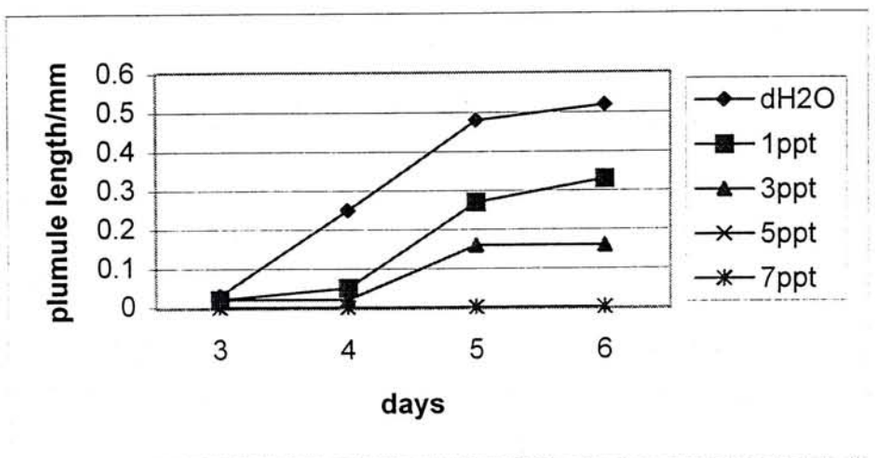

Fig. 3.08. Plumule lengths of seeds against days of treatment on different concentrations of NaCl at 12°C.

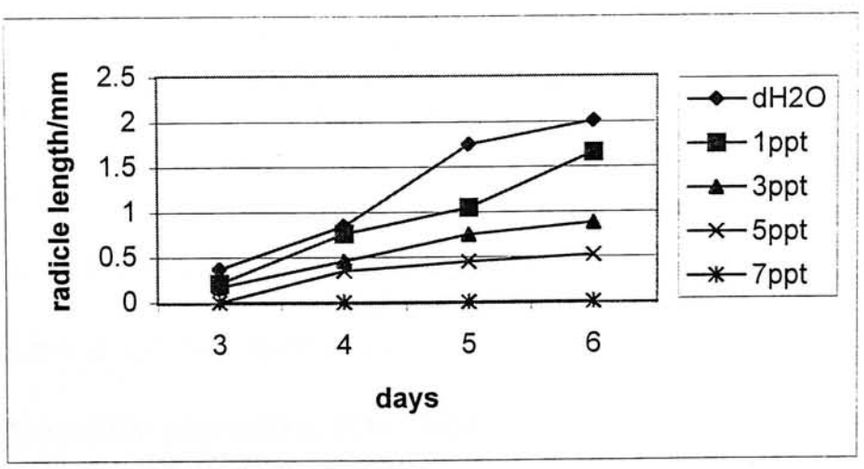

Fig. 3.09. Radicle lengths of seeds against days of treatment on different concentrations of NaCl at 12°C.

3.4 Discussions

In the experiment, mature seeds of *Mikania micrantha* should be careful selected, based on the following criteria. First, size of the seeds should be chosen, not too small or too large. Second, shapes of complete outline without defacets or any strange shapes were chosen. Then colour of the seeds should be dark black instead of the greenish-white or brownish in colour. Finally, the whole seed should be hard and tough on pressing.

Seeds of *Mikania micrantha* were treated with distilled water or different concentrations of sodium chloride. It was found that increasing concentrations of sodium chloride would decrease the percentage of seed germination and also decrease the plumule length and the radicle length of germinated seeds. It is because sodium chloride alters the nitrogen metabolism of the seed embryo axis (Bewley & Black, 1978; Rumbangh *et al.*, 1993). Hence failure in the making of building blocks of the seeds to germinate and hence the germination of seeds was retarded and the development in the building blocks of plumules and radicles were affected as well.

Increase in the concentrations of sodium chloride decreased the three parameters. It was found that for 1ppt, the effects produced on the percentage of seed germination, and plumule lengths and the radicle lengths were similar to that of the distilled water. For 3 ppt and 5 ppt onwards, a considerable inhibitory effects on these three parameters were observed. For 7 ppt, it was the minimum concentration of sodium chloride for the complete inhibition of the germination of the seeds.

For the germination of seeds for all concentrations of NaCl, similar patterns of increasing in the percentage of seed germination were observed. It was firstly a lag phase, followed by a log phase and finally a steady and decline phase. The lag phase was mainly due to the adaptation of the seeds to the surrounding environment, since it was subjected to the laboratory environment inside the petri dish. The log phase with a rapid rise in percentage since imbibition of water enabled the seeds to hydrolysed the stored foods to produce energy to build blocks in the seeds and hence faster rate of germination.

Since seed germination and subsequent growth of the plumule and radicle of *Mikania micrantha* were affected by sodium chloride, identification of the effective concentration of sodium chloride in this experiment (7 ppt), may suggest means to control the spread of the weed.

Chapter 4 : Sodium chloride as a herbicide for *Mikania micrantha* in Mai Po Marshes

4.1 Introduction:

Herbicide is defined as a chemical that can kill or inhibit the growth of some plants. They can be grouped into various categories based on their chemical similarity, mode of action (how they kill plants), herbicide movement within plants (mobile versus immobile), selectivity (selective versus nonselective), and application, and use patterns (Zimdahl, 1993).

On the selection of the use of herbicide, several considerations should be taken into consideration (Nalewaja, 1984):

(1) Will the herbicides control the weed species present?

(2) At what concentration (dosage, frequency and time) of the herbicide will it control the growth of weeds.

(3) Is the surrounding intermingling crops sufficiently tolerant of the herbicide?

(4) What is the danger of damage to nontarget plants? To prevent the aimless killing of other crops that are desirable for growth.

(5) Is the soil suited for the herbicide choice? Since the organic matter, clay content and pH can all affect the toxicity and persistence of herbicides. Usually, the toxicity is higher for clay and those soil low in organic matters.

(6) Are there other environmental dangers from the herbicide use? Selection of herbicides should be restricted to those that are neither prohibited from application near water nor extremely toxic to aquatic organism.

(7) Is the herbicide economic to use? It is assumed herbicide users will always weigh whether the potential increased economic return warrants herbicide use and also whether the herbicide is cheap.

4.2 Literature Review

4.2.1 Weed control

Weed control includes many techniques used to limit weed infestations and minimize competition. These techniques attempt to achieve a balance between cost of control and crop yield loss (Fryer, 1977; Floyd & Monaco, 1991; Zimdahl, 1993). There are several mechanical, non-mechanical, chemical and biological weed control techniques available and with its own advantages and disadvantages.

4.2.1.1 Mechanical control

Hand pulling and hand hoeing are good for annual weeds but not for perennials capable of vegetative reproduction because shoots partially removed from roots may then produce a new shoot (Buhler *et al.*, 1995). Both mechanical methods are labour intensive and inefficient. Tillage kills perennial weeds by physically damaging vegetative and reproductive parts of the plants. Finally, mowing removes shoot growth and thus prevents seed production and may deplete root reserves on some upright perennials. The best time to begin mowing is usually when the underground root reserves are at low level, between full leaf development and flower appearance (Nalewja, 1984).

4.2.1.2 Non-mechanical methods

Burning is a commonly used non-mechanical method. Burning must be repeated at frequent intervals if it is to control most perennial weeds.

4.2.1.3 Biological control

Biological control is defined as the action of parasites, predators or pathogens in controlling the population of weeds (Strobel, 1991).

Insect is a frequently used biological control agent. They may selectively destroy both the vegetative and the reproductive portions of weeds. Also they predispose weeds by causing diseases. Another type of pathogens frequently used are fungi. The repeated application of high levels of disease inoculum of fungi can cause the control of weeds (Barreto & Evans, 1995). Bacteria and viruses have also been successfully introduced to the weeds for the control (Evans & Ellison, 1990).

4.2.1.4 Chemical weed control

Use of chemicals that selectively kill weeds in crops is an integral part of many modern weed management systems (Fryer, 1977; Floyd & Monaco, 1991; Zimdahl, 1993). Herbicides can be selective which act on a particular plant or can be nonselective which have a broad spectrum of plants to be acted on. The use of herbicides is energy-efficient and economic. Herbicides eliminate and greatly reduce the need for hand weeding and hence reduction in labor and time needed for the weed control. However, the uses of herbicides are of potential problems which include the damage to the untarget plants, environmental pollution and toxic accumulations in the food chains such as fish and birds. In order to avoid the potential problems, proper use of herbicides should be made. Herbicide users should select the right herbicide for weed problem and crop, handle and store the herbicide with respect, and correctly apply the chemical.

4.2.2.1 Foliar active herbicides:

4.2.2.1.1 Leaf properties:

Since leaves are the principal point of entry for herbicides into plants, their structure and function are important. The primary leaf tissues are the epidermis, mesophyll, and vascular system. For herbicide entry, the thin epidermal layer is most important. It can be regarded as the first hurdle an herbicide molecule must cross to enter a plant. The epidermis is present on upper and lower leaf surfaces and consists of a single layer of interlocking cells. It is covered by cuticle that is often layered with waxes.

Plants with a thick, waxy cuticle layer absorb less herbicide or absorb the same amount more slowly than plants with a thin cuticle (Liu *et al.*, 1995). Leaves with hairs are usually trapping the vapours of the applied herbicides easily. Herbicides also enter plants through stomata. Although direct absorption by leaf surfaces is the most common route of entry, liquid spray droplets or volatile gases can enter stomata. Stomata are distributed on both upper and lower surfaces or only on the lower surface. Herbicides may more readily penetrate plants with many large stomata on the upper leaf surface than those with few or no stomata.

Surface acting agents (surfactants) which can lower the surface tension of water and

increase the wetting effect of herbicide are used in some herbicide formulations to assist entry (Nalewaja & Woznica, 1988; Silcox & Holloway, 1989; Holloway & Edgerton, 1992) and can often determine the amount of herbicidal activity obtained because of their influence on penetration of leaf surfaces.

4.2.2.1.2 Environmental factors:

Moisture is one of the important environmental factors favoring the translocation of herbicides. Lower humidity may create water stress which causes stomatal closure and hence delay in the transportation of herbicides. Light is essential for photosynthesis which is favored by the opening of stoma for gaseous exchange in the presence of light, hence favoring the translocation of herbicides. Warmth at intermediate temperature favors metabolic rate and physiological activity of plants, hence enhancing translocation activity of herbicides.

4.2.2.1.3 Foliage absorption:

This is the absorption of herbicides that are applied directly to the leaves of a plant. Its penetration can be stomatal penetration or cuticle penetration. The herbicide retention is not changed but foliage absorption and acropetal translocation is increased while

basipetal translocation is decreased on the application of herbicides (Liu *et al.*, 1995).

(a) Stomatal penetration:

Stomatal presence, exposure, and distribution vary among different plant species and among plants of the same species grown in different environments. Stomata are obvious ports of entry and stomatal openings vary under field conditions. Stomatal entry requires low surface tension and high wetting power and that is a difficult combination to obtain for a wide range of plants (Liu *et al.*, 1995).

(b) Cuticle penetration:

Cuticular penetration is often easier and carried out regardless of stomatal opening and occurs under a range of environmental conditions. The outer cell wall composition of plant are made of cutin, cutin wax, pectin and cellulose. Two routes by which exogeneous molecules may transverse the thickness of the cuticle surface into the living inner cells: a lipoid route and an aqueous pathway. Compounds such as dinitro and phenoxy compounds penetrate the cuticle in the lipid soluble form. Penetration is enhanced by formulation in weak acids such as esters or as salts of weak bases (Floyd & Monaco, 1991)

Compounds that enter via an aqueous route is moved more slowly and their penetration is greatly benefited by a saturated atmosphere .

4.2.2.2 Soil-applied herbicides:

These herbicides are usually applied into the soil of the growing plants to exert their effects on the growth of the plant.

4.2.2.2.1 Absorption from soil:

It is generally conceded that herbicides enter roots via root hairs and the symplastic system, the same pathway that inorganic ions (plant nutrients) follow. Passive and active uptake processes occur, but most herbicide uptake is passive with absorbed water, and movement, with water, is in the apoplast. Active uptake involves respiration energy, oxygen, entry into protoplasts and movement in the symplast.

4.2.2.3 Translocation:

Translocation of herbicides takes place through phloem and xylem, the transport systems in plants. There is a great deal of evidence to show a direct correlation between foliar uptake of herbicides and phloem transport; root uptake and xylem transport. There

are three routes of translocation: apoplast, symplast and apoplast-symplast (Zimdahl, 1993). Apoplast route involves movement exclusively in cell walls to the xylem. The symplast route involves initial entry into cell walls and then into the protoplasm of the cells. The apoplast-symplast route involves the re-entry of herbicide into the cell walls after bypassing the Casparian strips of endodermis. For translocation in the phloem, it is usually a movement from source to sink (from regions of high carbohydrate synthesis to regions of high use). Moreover, source is the point of entry of herbicides and the sink is the sites of high metabolic activity where herbicides express their toxicity.

4.2.3 Mode of action of herbicides:

Herbicides have three major mechanisms of action: (1) inhibition of respiration and photosynthesis, (2) inhibition of plant growth, and (3) inhibition of biosynthetic process (Floyd & Crafts, 1973).

For the inhibition of respiration, herbicides may act as an uncoupler for the oxidative phosphorylation. Uncoupling of oxidative phosphorylation is like braking while continuing to press the accelerator. Energy is released as electrons passing down the electron transport chain to oxygen and is trapped by converting ADP to ATP. If you

uncouple but keep the accelerator down, the motor will race and overheat. Also they may act as the inhibitors for the electron transport in the oxidative phosphorylation to produce ATP (Harwood, 1991).

As for photosynthesis, two photosynthetic light reactions are coupled by the photosynthetic electron transport chain where photophosphorylation (produce ATP) occurs. Light reaction I produces reduced nicotine adenine dinucleotide phosphate (NADP). Herbicides that act in relation to light reaction I short circuit electron transfer and generate toxic molecular species. Light reaction II begins with removal of electrons from water and production of oxygen (Hill reaction) and is where many herbicides exert their effect by blocking electron transport by binding to adjacent sites on the D-1 quinone protein, which functions in the electron transport chain between primary electron acceptor (Q) from chlorophyll a and plastiquinone (PQ).

For the inhibition of plant growth, some herbicides interfere with the function and transport of the natural auxin hormones (Floyd & Crafts, 1973). These hormones are useful to plants acting as the growth regulator to stimulate plant growth, particularly the coleoptile tissue. Also, they are responsible for the positive phototropism response of the shoot of plants under sunlight.

For the inhibition of the biosynthetic processes, they can be cell division or mitotic

inhibitors. One of the process is inhibition of carotenoid synthesis (Boger & Sandmann, 1992). Since carotenoids are essential to plant survival because they protect individual pigment-protein complexes and ultimately the chloroplast against photoxidation. With high light intensity or under stressed conditions, chlorophyll molecules receive more light energy than they can transfer effectively into electron transport. The excess energy can be given up in several ways including production of singlet oxygen that is destructive to tissue integrity. Carotenoids protect against this by quenching excited chlorophyll molecules and by quenching singlet oxygen. Destruction of carotenoids or their biosynthesis leads to loss of the protective role. Finally, the inhibitors of membrane structure may inhibit lipid biosynthesis in the membrane or directly disrupt the cell membrane.

Another process is the inhibition of chlorophyll biosynthesis inhibition (Harwood, 1991), since the first specific precursor for the formation of chlorophylls and other tetrapyrroles is α-aminolevulinic acid (ALA). Two pathways to ALA are thought to exist in plants. The first, the Shemin pathway, involves a pyridoxal phosphate-requiring condensation of succinyl-CoA and glycine and may be located in the mitochondrion. The second, the so-called C_5 pathway, is plastid-located and involves the transformation of glutamate to form ALA. The pathway is shown in Figure 4.01. Hence any block of herbicidal action on the ALA synthase or ALA dehydratase will cause the inhibition in chlorophyll synthesis.

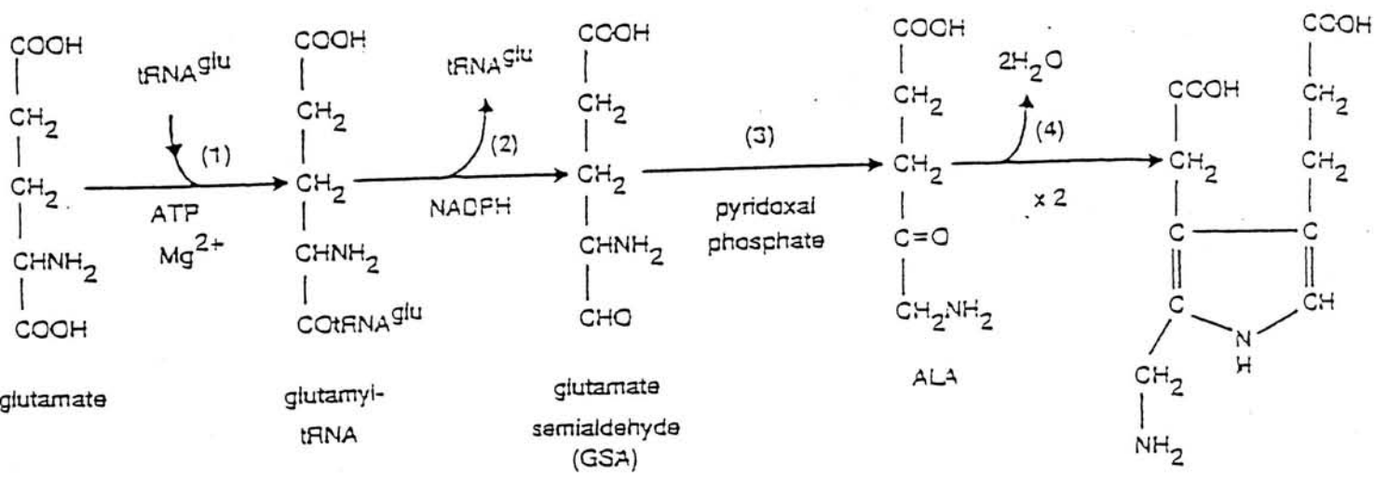

Fig. 4.01 Proposed biosynthetic sequence of ALA synthesis from glutamate to the condensation of two ALA molecules, forming porphobilinogen. The enzymes (1) glutamyl-tRNA synthetase, (2) glutamyl-tRNA dehydrogenase, (3) ALA synthase and (4) ALA dehydratase.

4.2.4 Surface-active agents (surfactants)

Surfactants are added to herbicides to increase the wetting effect of herbicides, to reduce the surface tension and increase penetrating power of herbicides (Kirwood, 1993). The range of formulation additives have been reviewed with particular reference to surfactants. Four classes of surface-active agents have been defined (Holloway & Stock, 1990):

(a) Anionic, in which the surface-active properties are provided by a negatively charged ion: a hydrophobic group is balanced by a negatively charged hydrophilic group.

(b) Cationic, in which the surfactant properties are provided by a positively charged ion: the hydrophobic group is balanced by a positively charged hydrophilic group.

(c) Nonionic, in which there are no electrical charges: the hydrophobic group consists of alkylphenols, fatty alcohols or fatty acids and is balanced by nonionized hydrophilic groups.

(d) amphoteric, having a molecular arrangement of hydrophilic groups which have the potential to become cationic in an acid medium or anionic in alkaline conditions.

Surfactants thus contain hydrophilic and lipophilic groups, and the balance between the opposing effects is called the hydrophilic-lipophilic balance (HLB). Compounds of low HLB are relatively lipophilic while those of high HLB are relatively water soluble. Thus, it is possible to select surfactants of certain HLB values for specific purposes.

Surfactants can be divided into two categories according to their application and mode of action (Silcox & Holloway, 1989). "Spray modifiers" reduce the surface tension of the spray droplets and are added to improve the wetting and spreading properties of formulations. They are particularly useful in the case of nonsystemic compounds, where uniform coverage of the canopy is important. "Activators" are added specifically to improve foliage absorption. Their activities depend upon specific physiocochemical interactions between the surfactant and the plant.

4.3 Materials and methods

The experiments were carried out in the greenhouse and the field. First the seeds *of Mikania micrantha* were pregerminated in petri dishes. It was left for five days for germination at room temperature. The germinated seeds with the complete and healthy plumule, radicle and cotyledons would be then chosen for the transfer to flower pots for growth of the seedlings.

The flower pots were 10.5 cm in height and 8 cm in diameter. The soil was made up of peat moss and perlite in the ratio of 3:1 was added to about 3/4 of the height of the flower pots. Then it was followed by watering the soil to make them ready for the transfer of the germinated seeds to the flower pots.

The germinated seeds were then transferred from the petri dishes to the flower pots. Two germinating seedlings were planted in each plastic pot. The radicles of the germinating seedlings were slightly pressed down into the soil and left the plumule and the cotyledons on top of the soil. Then the potted plants were then keep in greenhouses. The temperature was about 25°C-28°C and the photoperiod was 10-12 hours.

The plants were watered daily and nutrient solution with the following formula was added twice a week:

Stock solution	modified nutrient solution ML/L working soln.
1M $Ca(NO_3)_2$	4.5
1M KNO_3	5.5
1M $MgSO_4$	2.5
1M KH_2PO_4	1.25
0.0895M FeNaEDTA	1
1.25M NH_4NO_3	1
micronutrient soln.	1

The seedlings were left undisturbed and grown in the environment in greenhouse before experiment of the treatment.

4.3.1 Treatment of the seedlings of *Mikania micrantha*

4.3.1.1 Treatment solutions of NaCl

Saline solutions at the concentrations of 1%, 3% and 5% for the treatment of leaves *of Mikania micrantha* were prepared. In order to increase the wetting effect of NaCl on the leaf surface, the detergent of brand "Axe" was added to a concentration of 1.5% as the surfactant.

4.3.1.2 Foliage application

(a) Smearing by brushes:

Leaves of *Mikania micrantha* were chosen and labelled. To each leaf, NaCl of a certain concentration: 1%, 3% or 5% was smeared by brush onto the upper leaf surface. The direct opposite leaf was left untreated and used as the control for observations. Observations were made for the events or descriptions occurred in the leaf by a particular treatment of the leaf.

(b) Spraying by nozzles:

The leaves of *Mikania micrantha* were sprayed with the treatment solution of NaCl of a certain concentration: 1%, 3% or 5%. It was sprayed with a nozzle carefully so as not to spray into the soil to avoid direct damage to the root. Hence it was sprayed to the horizontal portion of the growing leaves on branches trailing away from the pots. Subsequent symptoms of the leaves after treatment with NaCl solution were recorded.

4.3.1.3 Soil-application of herbicide

For the pots containing the seedlings of *Mikania micrantha*, 7 ml of the prepared NaCl solution was added into the soil once daily for 10 days. These pots after treatment were left in the environmental conditions of photoperiod, temperature, humidity and wind velocity in greenhouse. Growth of seedlings were recorded and any changes produced on the plants were observed. The results of treatment were compared based on different concentrations of NaCl.

4.3.1.4 Floret-application of herbicide

For the open-site selected for the stable and steady growth of *Mikania micrantha*, florets were chosen to study the effect of NaCl and the site was set up to prevent disturbance. Then the florets were splashed with 5% NaCl once daily for 3 days. Any changes in the floret appearance and any changes in the colour and dryness of the florets.

4.3.1.5 Effect of different surfactants on growth *of Mikania micrantha* in foliage application of NaCl

Three surfactants were chosen: Lauryl sulphate (sodium dodecyl sulphate), Decon 90 and commercial product with brand "Axe". The concentration of each surfactant in the NaCl solution was 1.5%. It was made by adding 1.5 ml of surfactant to 100 ml of NaCl. Then the mixture solution was sprayed with nozzle separately into different pots of plants. They were sprayed to the leaves or branches trailing away from pots to avoid their effect on roots. The effects of the three different surfactants with NaCl on *Mikania micrantha* were studied. Also, the effects of each individual surfactant without NaCl sprayed onto the leaves were studied.

4.3.1.6 Effect of sodium chloride on the mangel plants

Sites with the growth of mangel plants were selected and set up. In the experiment, the mangel plants *Kandelia candel*(水筆仔) and *Aegiceras corniculatum*(桐花樹) were studied. These were the plants in Mai Po found to be affected by *Mikania micrantha*. Leaves of these plants were smeared with sodium chloride solution twice daily mixed with 1.5% detergent "Axe". The smeared leaves were then labelled for regular visits. The period of visits were from 9th to 27th February, 1999. Any symptomatic changes on the leaves were recorded.

4.4 Results

Sodium chloride which was used as the herbicide for the control of growth of *Mikania micrantha* was found to be effective in foliage-application, soil-application and floret-application.

4.4.1 Foliage application

For the treatment of the leaves of *Mikania micrantha* with sodium chloride solution of different concentrations, it was found that higher concentrations of sodium chloride expressed a stronger inhibitory effect on the growth of the leaves of *Mikania micrantha*. It was found that for 1% (applied twice daily), it took about 7 days for the leaves to become yellow and withered (Table 4.02). For 3%, it took about 5 days (Table 4.03) and for 5%, only 3-4 days (Table 4.04) for the yellowing and withering of leaves.

For the smearing experiment, leaves were treated with sodium chloride of a particular concentration. Ten sequence of events were shown in Table 4.01 and in Figures 4.02 to 4.08. The time sequence of the events of the leaves occurred in treatment of leaves of *Mikania micrantha* with 1%, 3% or 5% of NaCl solution were shown in Table 4.02, 4.03 and 4.04. The tips of leaves rolled up at both the 1% and 3% solution, followed by the flaccid of the leaf tip and then the flaccid of the margins. In treatment with 5% NaCl,

margins became flaccid was firstly observed. It was shown that the speed for the death of the leaves corresponded with the concentration of the NaCl solution, fastest in 5% and slowest in 1%. In some leaves, after the treatment with NaCl solution, necrosis occurred on the leaves. Then it was the blackening of the flaccid parts of leaves and finally the yellowing, withering of the whole plants. Usually the latter sequence of steps required longer time for occurrence.

The smearing experiments for the 3% and 5% NaCl treatment of 100 leaves of the *Mikania micrantha* were shown in Table 4.05 and 4.06. The Tables summarized the total number of the leaves that occurred with certain symptoms of defects of leaves. Also, the time sequence for the data collection of the 100 leaves for each treatment was recorded. The sequence of events from rolling up of leaf tips to death of leaf that occurred in the treatment groups with sodium chloride of different concentrations followed similar patterns but the timing for occurrence of the events was different. It was found that the sequence would be faster in the 5% NaCl treatment group than the 3% group. It was found that for 5% NaCl, it required about 4 days for the complete yellowing and death of leaves but it took about 6 days for 3% NaCl treatment to result in the yellowing and death of leaves.

For the spraying experiment of 5% NaCl onto the leaves of *Mikania micrantha* (Figure 4.09), approximately 50% of the leaves became yellow on day 3 of treatment and

complete yellowing on day 6 (Table 4.07). There was an increase in the percentage of yellowing of leaves with an increase in the number of days of treatment of the plant with sodium chloride solution.

However, plants showing yellowing of leaves due to NaCl were left undisturbed with continuous watering showed emergence of new leaves which were green and healthy. These newly formed leaves were continuously growing out from the stem. This indicated that short-term foliage application of NaCl caused the yellowing or death of the treated leaves but not the total death of the whole plant. Hence the effect was reversible after sometime. Therefore, it would be necessary to kill sufficient numbers of leaves on a plant and might also require repeated or periodical spraying with NaCl solution so as to deplete the food reserve and kill all the new leaves.

4.4.2 Soil-application

There was an increase in the percentage of leaf death in *Mikania micrantha* plants with the increasing concentration of NaCl treatment through soil application (Table 4.08). It was found that 5% NaCl would cause death in about 50% of the plants in 6 days of treatment whilst there was a total death on day 9. Whilst for 3% NaCl treatment, 50% death were found on day 8 whilst total death on day 11. For 1% NaCl, 50% death on day

11 whilst total death on day 15 of treatment. It was found that 5% NaCl had strongest effect in terms of time and effectiveness for killing the plant. Moreover, it is obvious that concentrations of 1%, 3% and 5% NaCl would exert killing effect on *Mikania micrantha*. Hence sodium chloride can be used as a soil-applied herbicide to control *Mikania micrantha*. The effects of soil-application of 5% and 3% NaCl to *Mikania micrantha* were shown in Figures 4.10 and 4.11.

It was found that when the plants were treated with soil-applied NaCl and were left undisturbed in the greenhousewith watering, there were no more emergence of new leaves and the whole plants died completely. Hence the systematic effect produced by the soil-applied NaCl on *Mikania micrantha* was found to be irreversible.

4.4.3 Floret-application

In the splashing of the florets of *Mikania micrantha* with 5% NaCl solution (Figure 4.12), it was found that firstly there was the drooping of the peduncle of the flowers, rendering the loss of integrity of hardness of peduncle at 5 hours after application of NaCl solution. It was then followed by drying and wilting of the flowers at 10 hours after application. The flower colour was found to be changed from white to yellow on day 1 and to brown on day 2 of the treatment (Table 4.09).

This application of NaCl to floret was found to be effective in killing the florets, since it produced significant damaging response to the flowers of *Mikania micrantha* in a short period of time.

4.4.4 Effect of different surfactants on growth of *Mikania micrantha* in foliage-application of sodium chloride

Mixture of NaCl with a surfactant, either lauryl sulphate (sodium dodecyl sulphate) or Decon 90 or the detergent of commercial brand "Axe", exerted similar damaging effect on the growth of *M. micrantha* (Table 4.10).

The three surfactants alone, however, caused no phytotoxicity or any yellowing of leaves after 15 days of treatment of leaves of *Mikania micrantha* by NaCl solution and the plants were found to be healthy (Table 4.10). The result was more obvious when compared with that of surfactants mixed with sodium chloride which caused yellowing of leaves from Day 2 onwards after treatment. Hence the phytotoxic effect of surfactants on leaves were considered negligible (Figure 4.13).

4.4.5 Effect of sodium chloride on the mangel plants

Smearing of sodium chloride solution onto the leaves of *Kandelia candel* and *Aegiceras corniculatum* caused no adverse symptomic expressions of leaves were found. For the sodium chloride solutions of concentrations of 1%, 3% and 5%, there were no symptoms of leaves nor any obvious damage over the 15 days of treatment (Table 4.11). The results of mangel plant *Kandelia candel* were shown in Figures 4.14 and 4.15 and those of *Aegiceras corniculatum* were shown in Figures 4.16 and 4.17. The results were sharply contrasting to the yellowed leaves of *Mikania micrantha* on Day 7, 5 and 3 of treatment with 1%, 3% and 5% sodium chloride solutions, respectively (Figure 4.09).

Table 4.01. Ten sequences of events of symptoms developed on leaves of *Mikania micrantha* after treatment with NaCl solution.

	Symptoms
1.	leaf tips roll up to 0.2-0.5cm
2.	leaf tips roll up to 0.5-1cm
3.	leaf tips flaccid
4.	leaf margin flaccid
5.	drooping of the leaves
6.	decreased in the sizes of leaves
7.	leaf necrosis
8.	flaccid part blackened
9.	whole leaf blackened
10.	yellowing /death of leaves

time	tip rolls up	tip	margin	drooping	decrease	leaf	flaccid part	whole leaf	yellowing
hours	(0.2-1cm)	flaccid	flaccid	of leaf	in size	necrosis	blackened	blackened	of leaf
1/2									
1	+								
2	+								
4	+								
6	+								
8		+							
10		+							
16		+	+						
18		+	+						
20			+						
22			+						
24					+				
26					+				
28					+	+			
30					+	+			
32					+	+	+		
34							+		
46							+		
48							+		
50							+		
52							+		
56							+		
70							+		
72								+	
76								+	
94								+	
96								+	
120									+
124									+
128									+
142									+
144									+

Table 4.02. The sequence of events of leaves of *Mikania micrantha* treated with 1% NaCl solution.

time	tip rolls up	tip	margin	drooping	decrease	leaf	flaccid part	whole leaf	yellowing
hours	(0.2-1cm)	flaccid	flaccid	of leaf	in size	necrosis	blackened	blackened	of leaf
1/2									
1	+								
2	+								
4	+								
6	+	+							
8		+							
10		+	+						
16			+						
18			+						
20					+				
22					+				
24					+	+	+		
26						+	+		
28						+	+		
30							+		
32							+		
34							+	+	
46								+	
48								+	
50								+	
52								+	
56								+	
70								+	
72								+	+
76									+
94									+
96									+
120									+
124									+
128									+
142									+
144									+

Table 4.03. The sequence of events of leaves of *Mikania micrantha* treated with 3% NaCl solution.

time	tip rolls up	tip	margin	drooping	decrease	leaf	flaccid part	whole leaf	yellowing
hours	(0.2-1cm)	flaccid	flaccid	of leaf	in size	necrosis	blackened	blackened	of leaf
1/2			+	+					
1			+	+					
2			+	+					
4			+	+					
6			+	+	+				
8					+		+		
10							+		
16							+		
18								+	
20								+	
22								+	
24								+	
26								+	
28								+	
30								+	
32								+	
34								+	
46									+
48									+
50									+
52									+
56									+
70									+
72									+
76									+
94									+
96									+
120									+
124									+
128									+
142									+
144									+

Table 4.04. The sequence of events of leaves of *Mikania micrantha* treated with 5% NaCl solution.

Treatment: 3%

symptoms	margin,tip	drooping	whole leaf	leaf margin	flaccid part	whole leaf	yellowing
time/hour	rolls up	of leaf	flaccid	rolls up	blackened	blackened	of leaf
1/2	21						
1	38						
2	42						
3	59						
4	68						
5	75						
6	78						
8	82						
10	84		22	20			
22			28	24	2		
24			36	29	8		
26			38	35	12		
28			47	42	15		
30			56	47	18		
32			59	52	22		
34			62	53	25		
46			68	54	29		
48			72	54	32		
50			79	54	37	4	
52			80	56	38	6	
56					46	25	
70					58	42	
72					60	40	
76					32	60	8
94					7	72	21
96						62	33
100						48	52
120						24	76
124						11	89
128						4	96
138						2	98
140						1	99
144							100

Table 4.05. The effect of 3% NaCl on the growth of leaves of *Mikania micrantha.* Numbers showing the number of leaves with the corresponding symptoms.

Treatment : 5%

symptoms	margin,tip	drooping	whole leaf	leaf margin	flaccid part	whole leaf	yellowing
time/hour	rolls up	of leaf	flaccid	rolls up	blackened	blackened	of leaf
1/2	38	63					
1	68	90					
2	72	98					
3	80	100	44	33	8		
4			56	35	10		
5			57	36	12		
6			60	38	12		
8			62	40	12		
10			63	40	15		
22			64	42	18		
24			68	45	20		
26			72	46	22		
28			78	46	24	4	
30			80	48	25	12	
32			88	48	28	18	
34					45	30	2
46					56	32	6
48					60	35	8
50					76	45	18
52					82	50	25
56					12	44	38
70					8	32	56
72						18	82
76						8	92
94						2	98
96							100

Table 4.06. The effect of 5% NaCl on the growth of leaves of *Mikania micrantha.* Numbers showing the number of leaves with the corresponding symptoms

Days	% of yellowing of leaves
1	0
2	35
3	50
4	60
5	75
6	100
7	100
8	100

Table 4.07. Effect of foliage application of 5% NaCl by spraying method on the growth of leaves of *Mikania micrantha*

	% of death of plants		
days	5%NaCl	3%NaCl	1%NaCl
1	0	0	0
2	0	0	0
3	0	0	0
4	10	2	0
5	15	3	0
6	50	5	0
7	80	20	0
8	95	50	15
9	100	75	20
10	100	80	35
11	100	100	50
12	100	100	70
13	100	100	85
14	100	100	90
15	100	100	100

Table 4.08.Effect of soil-application of 1%, 3% or 5% NaCl on the growth of *Mikania micrantha*

Hours	Description of the florets
5	drooping of the peduncle bearing the florets
10	inflorescence of florets dry and wilted
24	change of colour of florets from white to yellow
48	florets change to brown colour and complete dry

Table 4.09. Effect of floret splashing of NaCl solution on *Mikania micrantha*

Days	1	2	3	4	5	6	9	12	15
lauryl sulphate + 5% NaCl	+	++	+++	++++	++++	+++++	+++++	+++++	+++++
Decon 90 + 5% NaCl	+	+++	+++	++++	++++	+++++	+++++	+++++	+++++
"Axe" + 5% NaCl	+	++	+++	++++	++++	+++++	+++++	+++++	+++++
lauryl sulphate	---	---	---	---	---	---	---	---	---
Decon 90	---	---	---	---	---	---	---	---	---
"Axe"	---	---	---	---	---	---	---	---	---

"+" increasing severity in the yellowing of leaves of *Mikania micrantha*
"---" non-symptomic growth of leaves

Table 4.10. Effect of different surfactants with or without sodium chloride on foliage application to *Mikania micrantha*

	Any symptomatic damage to leaves		
Mangel plants	1%NaCl	3%NaCl	5%NaCl
Kandelia candel	No	No	No
Aegiceras corniculatum	No	No	No

Table 4.11. Effect of foliage-application of sodium chloride on mangel plants.

Fig.4.02. The rolling up of leaf tip of *Mikania micrantha* in treatment with foliage-application of 1% NaCl by smearing at Day 2.

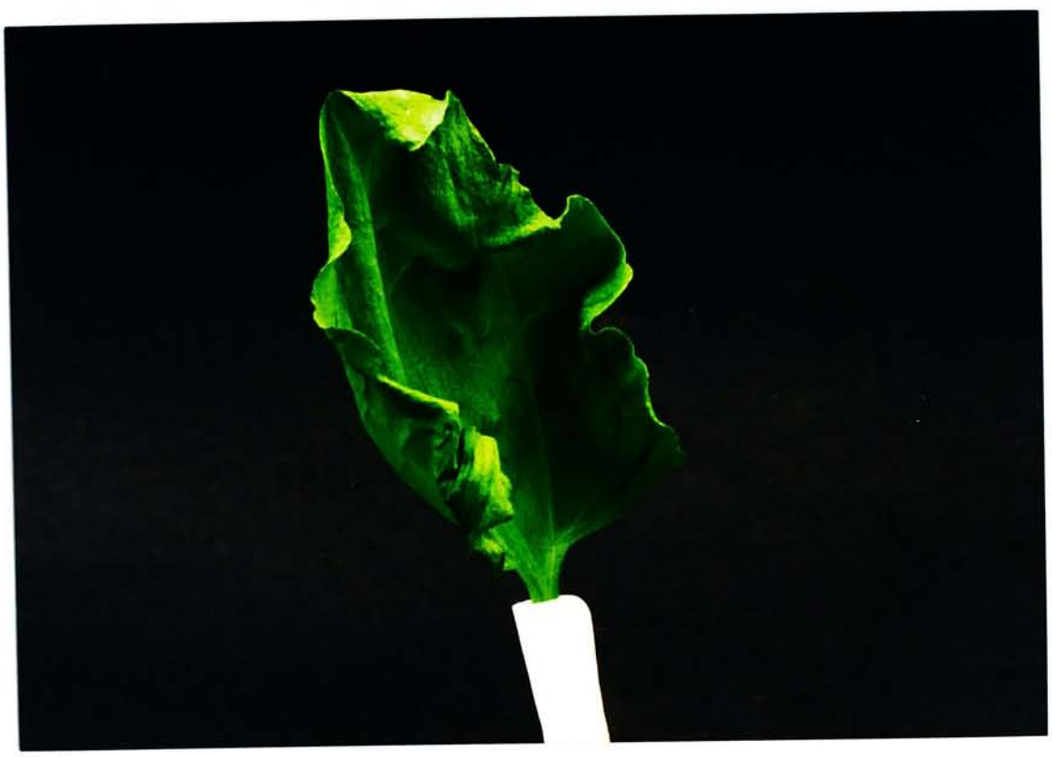

Fig. 4.03. The rolling up of the tip and margins of the leaf of *Mikania Micrantha* in treatment with foliage-application of 1% NaCl by smearing at Day 2.

Fig 4.04. The rolling up and becoming flaccid of the tip and margins of the leaf of *Mikania micrantha* in treatment with foliage-application of 1% NaCl by smearing at Day 3.

Fig. 4.05.The necrosis (brown regions on leaf) of leaf of *Mikania micrantha* in treatment with foliage-application of 1% NaCl by smearing at Day 3.

Fig. 4.06. The blackening of the flaccid parts of leaf of *Mikania micrantha* in treatment with foliage-application of 1% NaCl by smearing at Day 4.

Fig. 4.07. The blackening of the whole leaf of *Mikania micrantha* in the treatment with foliage-application of 1% NaCl by smearing at Day 5.

Fig. 4.08. The yellowing and death of leaf of *Mikania micrantha* in treatment with foliage-application of 1% NaCl by smearing at Day 6.

(a)

(b)

Fig. 4.09. The treatment of leaves of *Mikania micrantha* by spraying with 5% NaCl (a) before the spraying and (b) on Day 5 of spraying.

Fig. 4.10. The effect of soil-application of 5%NaCl to *Mikania micrantha* on Day 6 of treatment.

(a)

(b)

Fig. 4.11. The effect of soil-application of 3%NaCl to *Mikania micrantha* (a) before the treatment and (b) on Day 6 of treatment.

(a)

(b)

Fig. 4.12. The effect of floret-splashing of 5% NaCl on the florets of *Mikania micrantha* (a) before the splashing and (b) on Day 2 of splashing.

(a)

(b)

Fig. 4.13. Effect of surfactants: lauryl sulphate, Decon 90 and "Axe" on leaves of *Mikania micrantha*. (a)alone at Day 15 and (b) mixed with 5% NaCl at Day 3 of treatment.

Fig. 4.14. Natural growth of mangel plant *Kandelia candel*.

(a)

(b)

Fig. 4.15. Treatment of leaves of *Kandelia candel* by smearing with (a) 5% NaCl and (b) 1% NaCl at Day 15.

Fig. 4.16. Natural growth of mangel plant *Aegiceras corniculatum*.

(a)

(b)

Fig. 4.17. Treatment of leaves of *Aegiceras corniculatum* by smearing with (a) 5% NaCl and (b) 1% NaCl at Day 15.

4.5 Discussions:

Sodium chloride was found effectively acting as a herbicide for the control of *Mikania micrantha* by various routes of application.

(a) Foliage-application of NaCl

In the treatment of leaves *of Mikania micrantha* with NaCl solution, there were the development of sequence of events leading to the symptoms expressed by the leaves. Since the concentration of NaCl is hypertonic to that of cell sap of the leaves of *Mikania micrantha*, water would move from the cell sap of the leaf cells to outside of the leaves through the selectively permeable membrane. NaCl would cause a loss of water from the leaves of the plants. Hence firstly there will be the drooping of the petioles of the leaves due to water loss in the 5% treatment group but it is not shown for 1% and 3% groups, since the effect was slower and not obvious. The rolling up of the leaves can be explained by the fact that different parts of leaf surface lose water at different rates, hence causing the curving of the leaf structures particularly the thinner leaf tip and leaf margins with fewer cell layers and little vascular support.

Then it was followed by the flaccid of the leaves, mainly due to more water loss due to osmotic effect throughout the leaves. Moreover, the loss of water will cause the wrinkling of the leaves and hence a decrease in the size of leaves were observed.

Sometimes there will be some necrosis (death of parts of the tissues in plants usually in a spotted-way), due to excessive water loss in some tissues leading to the breakdown of cellular metabolism such as phtosynthesis, respiration or nucleic acid and protein synthesis. Usually the necrosis appeared as the brownish-spots located mainly in the interveinal regions of the leaves.

The flaccid parts of the leaves would then be blackened, mainly because chloroplasts in the leaves had been destroyed. Hence the flaccid parts changed from green colour to black colour. This occurred when the NaCl acts as a herbicide to exert its effect on the biosynthetic processes. The inhibition in the biosynthetic processes in chlorophyll formation will render the loss of green pigment. Hence blackening occurred. These blackening of leaves spreaded first from the flaccid parts to other parts of leaves until the whole leaves became blackened.

Finally, the leaves changed to yellow in colour and withered to death. These may be explained by the fact that the failure of the photosynthesis of the plants, rendering the plants suffered from the shortage of food. Also the loss of water would cause the inhibition of metabolic activities in plants. Hence the leaves became yellow and finally died.

For the translocation of the NaCl to the leaves, it is probably through the stoma of the

leaves due to the wetting agent (surfactant) which helped in the retention of NaCl on the leaf surface. The translocation is then through the apoplast and then symplast to the phloem of the plant. NaCl is redistributed on the leaf surface and then NaCl may be translocated basipetally (from top to base), to other parts of the shoot and the roots of the plants.

(b) Soil-application of NaCl

For the soil-applied NaCl used as a herbicide, there were the death of the whole *Mikania* plant after a short period of treatment. It may be due to the systematic effects produced by NaCl on the roots of the plants. Since NaCl at concentrations of 1%, 3% and 5% are hypertonic to that of the cell sap of the root cells, loss of water from the root cells would cause water deficiency in the plant and hence inhibition of metabolism leading to the death of the plants. It is also possible that the systematic translocation of NaCl to the leaves of the plants causes the toxic effect of NaCl on the leaves.

For comparing the effects of foliage-application and soil-application, it was found that the effect of foliage application was faster than that of soil-application. This can be explained by the fact that the soil-application requires the systematic translocation of the NaCl which requires a longer time for the translocation of NaCl from the root to the

leaves of the plant. Whilst for the foliage application, it is a more direct means for penetration of NaCl to produce the toxic effect on leaves. However, the damaging effect of foliage application to the plant was a reversible one; the plants suffering with the yellowing and death of the leaves were not dead and they could still produce new leaves. The effect of foliage application of NaCl was local and there would be little systematic effect. However, the effect of soil-application of NaCl was irreversible; there was the complete death of the plants and no more growth of new leaves from the plant. Hence the effect of soil application of NaCl was more profound, systematic and complete. Furthermore, in balancing the pros and cons of the foliage and soil-applied NaCl, we can choose different combinations of the two application methods for the control of *Mikania micrantha* in Mai Po Marshes.

The effect of adding surfactants to sodium chloride solution can increase the wetting effect and retention time for the sodium chloride and hence increase the control effect on *Mikania micrantha*. It was found that the surfactants themselves caused no phytotoxic effect to the weed and can be mixed with sodium chloride for the control of *Mikania micrantha*. The effect of the surfactant on the ecological environment in Mai Po Marshes, however, was not assessed.

The selectivity of the herbicide is important in controlling the weed. Hence the absence in negative response to NaCl in leaves of the mangel plants which are the

untarget plants for the spraying of sodium chloride is found to be significant. The use of NaCl to control *Mikania micrantha* in the mangrove areas in Mai Po would not do damage to other desired plants in the mangrove areas.

Chapter 5: Conclusions

Mikania micrantha is a vigorous weed. Its colonization is rapid and widespread. Its success may be accounted for by its mode of reproduction. In *Mikania micrantha*, its mode of reproduction was found to be apomixis (Section 2.4.1), since the experimental results of the apomixis one was similar to that of the untreated one. Apomixis is the formation of embryo without syngamy. It is an energy saving process in a plant since the formation of embryo does not require the formation of male and female gametes as well as their fusion. Also, the vigorous character of the plants can be passed onto the offsprings.

The control of *Mikania micrantha* targeting on the mode of reproduction can be possible by introducing sterility to the plants so as to prevent flowering or fruiting in *Mikania* by spraying herbicide on the flowers during the flowering season.

Germination of seeds of *Mikania micrantha* given water showed 80% germination. The germination of seeds given 3 ppt and 5 ppt was 52% and 35% (Section 3.3.1). Hence the sodium chloride concentrations showed a negative relationship with the percentage of seed germination. The higher the concentration of sodium chloride, the lower the percentage of seed germination. It was found that
7 ppt of sodium chloride caused complete inhibition of seed germination, and hence it could be used as a control agent to prevent *Mikania* germination.

The plumule and radicle lengths of *Mikania micrantha* were negatively related to the concentration of sodium chloride. The higher the concentration of sodium

chloride, the shorter would be the length of the plumules and radicles.

Sodium chloride at concentration 7 ppt (Table 3.01,3.02 & 3.03) marked the point where the concentration could cause the complete inhibition of seeds of *Mikania micrantha*. Hence any concentrations of sodium chloride higher than 7 ppt would cause the complete inhibition of seed germination.

In the use of sodium chloride as a herbicide in controlling the growth of *Mikania micrantha*, three routes of application to plants were tested. They were foliage-application (Section 4.3.1.2), soil-application (Section 4.3.1.3) and flower-splashing (Section 4.3.1.4).

The foliage application of sodium chloride involved the smearing and spraying methods, in which the concentration of sodium chloride used were 1%, 3% and 5%. Adverse reaction to NaCl in the tested leaves was obvious, including tip rolling, margin becoming flaccid, drooping of leaf petiole, decrease in leaf size, blackening of leaf, and finally the yellowing and death of leaf. The symptoms appeared in a sequence of time and followed an order of occurrence. For 1%, 3% and 5%, the pattern of occurrence of sequence of events were similar but with 5%, the sequence of events started at earlier time and proceeded faster. The yellowing of leaves of *Mikania micrantha* occurred at days 7, 5 and 3 of treatment with sodium chloride of concentrations 1%, 3% and 5% respectively (Section 4.4.1). For the foliage application of NaCl solution, the damage to the plants were reversible since there were the emergence of new leaves which were healthy and growth of the plants continued.

Soil application of sodium chloride involved the adding of sodium chloride solution to the soil of the plants and this involved the root damage as well as the death of whole plant. In the experiment, sodium chloride of concentrations 1%, 3% and 5% were used. It was found that the whole plant of *Mikania micrantha* died on days 12, 9 and 7 of treatment with sodium chloride of concentration 1%, 3% and 5% respectively (Section 4.4.2). The effect on the plants were irreversible. There was no emergence of new leaves and no further growth in the plants tested. The whole plants died and hence control of the plants would be possible.

The use of sodium chloride when applied to leaves and to roots, have pros and cons. For the leaf treatment, the leaf death would not seriously affect the whole plant since growth of the plants continued and there were the emergence of new leaves. In order to have complete control, regular spraying is required. For the soil application of NaCl, this involved the systematic damaging effect to the whole plant; hence it took longer time for the wilting of the whole plant. Plant death caused by NaCl was irreversible. However direct adding of sodium chloride to the soil will alter the sodium chloride concentration in the soil; hence use with care should be observed. In order to have a complete and effective control of *Mikania micrantha*, balancing the benefits of routes of application, different combinations of foliage-application and soil-application methods can be used.

Moreover, sodium chloride was found to be effective on flower splashing (Section 4.4.3). For the flower splashing, there was the drooping of the peduncle of the inflorescence of flower; then the flowers changed form white colour to yellow colour

and finally to brown, and drying and wilting occurred. It was found effective for sodium chloride to cause the above symptoms within 48 hours after the splashing. Since the response was so rapid and significant, the use of sodium chloride splashing to flowers would kill the flowers and formation of fruits.

In the treatment of leaves of *Mikania micrantha* with sodium chloride of different concentrations, detergent of brand "Axe" was added as a surfactant for increasing the retention of sodium chloride on leaf surface and improving the foliage absorption of sodium chloride. It was found that the three surfactants: lauryl sulphate, Decon 90 and "Axe" performed similar actions when mixed with sodium chloride in causing the wilting of leaves (Section 4.4.4). Experimental work demonstrated that lauryl sulphate, Decon 90 and "Axe" did not have phytotoxic effect on plants when applied alone without sodium chloride.

Mai Po is a natural reserve area for the natural mangrove vegetations and migratory birds. The spreading of *Mikania micrantha* to this area is rapid. *Mikania micrantha* is found on roadsides, fences and intermingling among many vegetations. The spreading of Mikania micrantha along the bunds of Gei Wai is rapid and wide (Section 1.4). Mangrove areas including the mangel plants of *Kandelia candel* and *Aegiceras corniculatum* were found to be intermingling with *Mikania micrantha.* Hence the problems of growth of *Mikania micrantha* need to be solved and local action is required. In this series of experimental studies, the use of sodium chloride as a herbicide showed possible value for controlling the growth of *Mikania micrantha* in the mangrove areas. Since the sodium chloride is a component in the natural habitat of mangrove area, it is not expected to cause serious disturbance in the ecology and

growth of the the mangel plants.

The spreading of *Mikania micrantha* to the mangrove area affect the mangel plants, *Kandelia candel* and *Aegiceras corniculatum*. Sodium chloride of concentrations 1%, 3% and 5% were sprayed onto the leaves of *Kandelia candel* and *Aegiceras corniculatum* during the experimental periods. These mangel plants were found to be healthy after spraying. Sodium chloride had demonstrated no phytotoxic effect on the mangel plants (Section 4.4.5). Hence sodium chloride can be used as a herbicide selectively aiming at just killing *Mikania micrantha* but not the mangel plants.

As a conclusion, *Mikania micrantha* showed good promise as a herbicide in controlling the growth of *Mikania micrantha* in Mai Po Marshes and possibly other mangrove areas.

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